INTRODUCTION

In the spring of 1951 Wolfgang BARGMANN suggested to Ernst and Berta SCHARRER that a symposium on neurosecretion should be organized to bring together investigators working in this field and thus provide an opportunity for the exchange of ideas. Inasmuch as the Stazione Zoologica at Naples had been host to three symposia [1] since the end of the war, Drs. Reinhard and Peter DOHRN were asked whether a symposium on neurosecretion might be held in the spring of 1953 as the fourth of the Naples Symposia. They approved and offered the facilities of the Stazione for such a meeting.

Originally it was thought that, as in former symposia at Naples, the number of participants would be between 10 and 15. However, the program finally contained 28 papers, almost all of which turned out to be rather extensive. Obviously, the publication of all papers in full in a supplement volume of the "Pubblicazioni," as it had been done in the case of the preceding symposia, would have severely strained the resources of the Stazione [2] and could not have been accomplished without considerable delay. It was decided, therefore, to make available a record of the proceedings in the form of abstracts, and to leave it to every participant to publish his paper in a journal of his choice. In these abstracts, reference is made to the papers in which the material presented at the symposium has been or will be published. Thus, the reader may get, from this collection of abstracts, an over-all idea of the present status of research in this new field; if he is interested in any particular aspect, the references following some of the abstracts should suffice to serve as a guide in the search for additional literature.

We speak for all who participated in the symposium, either actively by presenting papers and demonstrations, or as observers, when we express our sincere gratitude to the director of the Stazione Zoologica and his staff for having made the meeting possible by their cooperation and by many acts of thoughtfulness in matters large and small. A meeting of this kind is successful only if many people contribute time and labor. To all of them go our heartfelt thanks.

A number of participants received travel grants from various agencies in their respective countries. This symposium meant a great deal to those who were thus enabled to participate, and the support that anyone received was appreciated by all. In this spirit we wish to express our gratitude to those agencies which assisted individual participants. In particular, thanks are due to the Rask-Oersted Foundation and the University of Copenhagen (Denmark), the Royal Society of London (England), the Office des Relations Culturelles du Ministere des Affaires Etrangeres, Paris, the Faculte des Sciences, Paris, and the Universite de Bordeaux (France), the Bundesinnenministerium of the West German Government, Bonn, and the University of Kiel (Germany), the Consiglio Nazionale delle Ricerche, Rome (Italy), the National Science Council of Japan, Tokyo (Japan), the Swedish Natural Science Research Council, Stockholm (Sweden), the National Science Foundation, Washington, D.C., the American Cancer Society, New York, N. Y. and the University of Colorado (USA).

To our regret, Lewis H. KLEINHOLZ, Reed College, Portland, Oregon, USA, who was scheduled to give a historical survey of the investigation of the neurosecretory system in crustaceans, was prevented from participating in the symposium for reasons of health.

1
Symposium on Embryology and Genetics. Supplement to Vol. 21 of the Pubbl. Staz. Zool. Napoli. 1949 (238 pages)

Symposium on Mutagenics. Supplement to Vol. 22 of the Pubbl. Staz. Zool. Napoli. 1950 (200 pages, 8 plates).

Symposium on Submicroscopic Structure of Protoplasm. Supplement to Vol. 23 of the Pubbl. Staz. Zool. Napoli. 1951 (208 pages, 20 plates).

2
A grant-in-aid toward the Symposium was requested from UNESCO-IUBS, but was not obtained.

No.

J. Benoit & I. Assenmacher

(Laboratoire d'Histophysiologie du Collège de France, Paris, France)

Rapport entre la stimulation sexuelle préhypophysaire et la neurosécrétion chez l'Oiseau.

Les rayons lumineux stimulent fortement le développement des glandes génitales chez la plupart des Oiseaux lorsqu'ils agissent sur des animaux prépubères ou des adultes en période de repos sexuel. Chez le Canard, les radiations lumineuses, pénétrant dans les tissus de la région orbitaire, excitent deux chainons, superficiel et profond, d'une voie nerveuse qui s'étend de l'oeil à l'hypothalamus: la rétine et l'hypothalamus lui-même. Comment cette excitation nerveuse communique-t-elle à la préhypophyse, pour lui faire sécréter ses hormones gonadotropes, la stimulation nécessaire? La rareté des fibres nerveuses contenues dans cette préhypophyse (pars distalis), l'absence de fibres nerveuses se rendant de l'hypothalamus à la pars distalis font renoncer a une théorie purement nerveuse qui assurerait, par la seule voie des nerfs, la transmission directe de l'excitation lumineuse aux cellules glandulaires de l'hypophyse.

Les dispositifs vasculaires et nerveux que nous avons

observés au niveau de l'hypophyse et de l'éminence médiane du Canard suggèrent l'existence possible d'un mecanisme neuro-humoral. En effet, la vascularisation de la <u>pars distalis</u> est fournie presque complètement et parfois complètement par deux douzaines environ des veines portes qui drainent le sang d'un riche réseau capillaire étendu à la surface de l'éminence médiane. Ce réseau capillaire "sous-tubéral" (il est recouvert par des cordons de la <u>pars tuberalis</u>) est lui-même alimenté par les artères carotides internes. Ces veines portes sont groupées en un faisceau qui contient aussi des cordons cellulaires appartenant à la <u>"pars tuberalis"</u>. Ce faisceau, ou "tractus porto-tubéral", disposé entre l'éminence médiane et le hile préhypophysaire, est situé à deux millimètres environ en avant de la tige infundibulaire. Quelques veines portes, que nous appelons "postérieures", existent en outre derrière le tractus, entre ce dernier et la tige infundibulaire. Elles relient la région postérieure de l'éminence médiane et la <u>pars distalis</u>. Cette disposition générale permet la section chirurgicale isolée, soit d'un nombre plus ou moins considérable des veines portes, d'avant en arrière, soit de la tige.

La presque totalité ou la totalité du sang qui irrigue la <u>pars distalis</u> a donc préalablement circulé à la surface de l'éminence médiane. Or celle-ci présente, entre le chiasma et le faisceau des veines portes, une région particulière, qui

sera drainée par les veines portes les plus antérieures, celles qui se rendent principalement à la partie antérieure de la <u>pars distalis</u>. Cette région rétrochiasmatique contient, dans sa couche superficielle, des dispositifs nerveux très remarquables: de nombreuses fibres nerveuses provenant de noyaux hypothalamiques, non encore précisés exactement, se dirigeant vers la surface de l'éminence, au milieu d'une quantité innombrable de prolongements névrogliques de cellules épendymaires, et se recourbent en anses très aigües à quelques microns ou fragments de micron des capillaires dont le sang se rendra ensuite à la <u>pars distalis</u>. En outre, la coloration à l'hématoxyline chromique-phloxine de Gomori révèle dans cette couche superficielle de très nombreuses et fines gouttelettes d'une substance neurosécrétoire semblable à celle que l'on observe dans les neurones des noyaux supraoptique et paraventriculaire, de même que le long du trajet du faisceau hypothalamo-hypophysaire qui se rend au lobe infundibulaire et dans ce lobe lui-même. Les images microscopiques suggèrent l'idée qu'une substance chimique pourrait se trouver dans la substance neurosécrétée par des neurones hypothalamiques et passer dans le sang du réseau capillaire superficiel de l'éminence médiane et ainsi dans la <u>pars distalis</u> dont elle stimulerait l'activité. L'hypothèse d'un message humoral transmis de l'hypothalamus à la <u>pars distalis</u> de l'hypophyse a déjà été émise par <u>Green</u> et <u>Harris</u> en 1947.

Pour soumettre cette hypothèse au contrôle expérimental~~e~~, nous avons fait subir à des Canards mâles impubères les trois opérations suivantes: 1) la section de la tige infundibulaire ou "mischotomie" (μισχος = tige); 2) l' "éminentiotomie" ou section transversale, au dessous du chiasma, de l'éminence médiane, c'est à dire, à son niveau, du faisceau hypothalamo-hypophysaire; 3) la "tractotomie" ou section, plus ou moins complète, du tractus porto-tubéral contenant les veines portes. Après ces expériences, les sujets furent, passé le délai de la régression testiculaire saisonnière normale et de la période réfractaire consécutive, exposés à la lumière artificielle pendant un mois environ. Voici les résultats généraux de ces expériences contrôlées par l'examen de coupes sériées pratiquées à travers toute la région hypothalamo-hypophysaire.

1) <u>Mischotomie</u>. La section de la tige fut réussie dans 5 cas. Le lobe infundibulaire était atrophique, très pauvre en substance neurosécrétoire colorable par l'hématoxyline de Gomori. La <u>pars distalis</u> était normale. Les testicules, très volumineux, pesaient 53, 68, 80, 85 et 89,5 gr. Evalués par planimétrie des coupes microscopiques, les volumes des <u>pars distalis</u> étaient, chez les 4 premiers sujets approximativement de 4,77 - 5,9 - 3,4 et 5,62 mm^3 (contre 4,57 - 5,08 - 5,6 et 6,15 mm^3 chez 4 sujets normaux) (1).

2) Eminentiotomie haute. Chez 5 sujets, le faisceau hypothalamo-hypophysaire fut interrompu, la substance colorée par l'hématoxyline chromique disparut en aval et s'accumula en amont de la lésion. Le lobe infundibulaire est atrophié. Les veines portes existantes drainaient encore du sang à la pars distalis, mais un sang ayant circulé à la surface d'une région de l'éminence dépourvue du fait de l'opération d'anses nerveuses et de substance qui se colore électivement par l'hématoxyline de Gomori. La portion antérieure (lobe céphalique) de la pars distalis présente une petite zone centrale atrophique. Le reste de la glande est formé de cordons cellulaires bien vascularisés et bien vivants. Les pars distalis ont, chez les 4 derniers sujets , d'après les coupes, un volume approximatif de 2,53 - 2,8 - 3 et 3,37 mmm^3, après soustraction du volume des zones atrophiques. Les testicules sont très petits et pèsent 0,4 - 0,7 - 1,03 - 1,5 et 2,22 gr. Les tubes sexuels sont au repos complet (2 cas) ou contiennent quelques rares spermatocytes (3 cas). (Chez 5 sujets incomplètement opérés de la même manière, les testicules pesaient 22, 41, 48, 68 et 72 gr.; les volumes de leur pars distalis furent évalués à 3,44 - 3,12 - 5,15 - 3,08 et 6,12 mm^3 de parenchyme sain).

3) Tractotomie. Nous exposons les résultats obtenus chez 4 sujets qui, malgré un éclairement artificiel de 1 mois, présentèrent à l'autopsie de petits testicules: 0,58 - 0,81 - 1,08 et 2,82 gr. Histologiquement, ces organes

contiennent des cellules interstitielles inconditionnées et des tubes séminaux en activité nulle ou très faible (quelques rares spermatocytes). Les pars distalis de ces sujets présentent, après fixation et coloration, des volumes évalués respectivement à 3,91 - 3 - 3,88 et 2,65 mm^3, après soustraction des petites zones atrophiques observées dans leur partie antérieure.(Chez 9 sujets incomplètement opérés et pourvus de testicules volumineux : 38 - 46,1 - 50 - 55 - 72 - 81 - 86 - 90 - 94 gr, les pars distalis avaient respectivement un volume de 5,86 - 4,92 - 4,07 - 2,53 - 6,34 - 5,03 - 4,56 - 4,04 et 5,58 mm^3). Ces poids concernent donc des parenchymes de cellules bien vivantes, et suffisamment vascularisées. Les vaisseaux qui irriguent ces préhypophyses sont des veines et en grande majorité des veines portes: outre les veines portes "postérieures", généralement ménagées par l'opération, on observe quelques veines portes du tractus porto-tubéral, soit que celui-ci n'ait été sectionné qu'incomplètement, soit que des veines se soient régénérées après l'opération. On observe aussi parfois des vaisseaux banaux, qui, issus des organes environnants, se sont mis en relation avec le hile de la pars distalis. Celle-ci continue donc à recevoir du sang, mais l'examen des coupes sériées permet de dire que ce sang ne provient plus de la région particulière rétrochiasmatique de l'éminence médiane riche en anses nerveuses et en substance neurosécrétée colorée par l'hématoxyline chromique. Il provient

soit de veines portes issues des régions de l'éminence médiane normalement dépourvues de ces différenciations spéciales, soit d'organes voisins non spécifiques.

Ces résultats nous suggèrent l'hypothèse suivante: Dans les conditions normales, l'activité gonadotrope de l'hypophyse impliquerait l'intervention d'une substance inductrice issue de certains noyaux hypothalamiques et contenue dans la substance neurosécrétée colorable par l'hématoxyline de Gomori, abondamment distribuée dans la région rétrochiasmatique de l'éminence médiane, entre le chiasma et le tractus porto-tubéral. Cette substance passerait normalement dans le réseau capillaire sous-tubéral qui recouvre l'éminence médiane et serait transportée par les veines portes à la *pars distalis*. Dans nos expériences, la section des seules veines portes qui drainent cette zone, c'est à dire la section des veines portes antérieures du tractus porto-tubéral, ou celle d'une partie du réseau capillaire sous-tubéral chez les éminentiotomisées, empêcherait la substance inductrice d'exercer son action sur la préhypophyse, et celle-ci n'accomplirait plus ou que très incomplètement sa fonction gonadotrope.

Peut-on objecter que ces lésions ont engendré, par ischémie, une atrophie partielle de la *pars distalis* et que cette dernière est devenue *quantitativement* insuffisante pour conditionner les gonades? Cette atrophie existe

incontestablement, mais elle ne paraît pas assez importante pour justifier cette critique. Le parenchyme restant serait, par son volume, largement capable de conditionner un développement testiculaire même complet s'il était fonctionellement normal. Nous pouvons dire en effet que, dans des cas d'hypophysectomie incomplète, chez le Canard, un reliquat égal au cinquième d'une glande normale suffit à conditionner un développement très important des testicules (Benoit, 1937). D'ailleurs, la comparaison des volumes des *pars distalis* des opérés et des témoins montre bien que la notion de volume ne doit pas intervenir. L'état des thyroïdes permet en outre de répondre à l'objection faite. Ces glandes endocrines présentent en effet dans la plupart des cas la structure d'organes dont l'activité paraît normale ou un peu diminuée, mais toujours supérieure à celle, nulle ou très faible, que l'on observe après hypophysectomie. La tractotomie et l'éminentiotomie ont donc entraîné la disparition totale ou presque complète de la fonction gonadostimulante de la préhypophyse, mais non celle de la fonction thyréotrope (2). La disparition de la fonction gonadotrope ne semble donc pas relever d'un phénomène d'ischémie.

Cette disparition de la fonction gonadotrope que nous observons dans nos expériences ne résulte pas davantage du fait que l'atrophie partielle de la *pars distalis* affecte

spécialement son lobe céphalique, et la fonction gonadotrope n'est pas localisée dans ce lobe. Des implantations séparées de portions céphaliques et caudales de préhypophyses de Canards normaux à des Souris femelles impubères nous ont montré en effet que les portions caudales étaient, comme les portions céphaliques, riches en hormones gonadotropes.

Il nous semble donc, jusqu'à plus ample informé, que le jeu normal de la fonction gonadotrope de la pars distalis est lié à l'intégrité de la zone rétrochiasmatique de l'éminence médiane où se trouve la substance colorable par l'hématoxyline de Gomori et de la circulation porte qui la relie à la préhypophyse.

(1) Ces chiffres, nettement inférieurs à ceux des volumes des préhypophyses fraîches (10 à 15 mm^3), s'expliquent par la rétraction occasionnée par le traitement histologique des pièces (inclusion à la paraffine).

(2) Nous ne pouvons pas encore expliquer aujourd'hui ce maintien de la fonction thyréotrope.

Le mémoire in extenso paraîtra dans les Archives d'Anatomie microscopique et de Morphologie experimentale (Masson, Editeur, Paris).

Pour information additionelle à voir les oeuvres suivantes des auteurs:

Assenmacher, I. - 1952 -

Arch. Anat. microsc. et Morph. exper. Vol. 41, p. 69.

Assenmacher, I. et Benoit, J. - 1953 - Contribution à l'étude des relations de la substance Gomori-positive avec le complexe hypophysaire et la gonadostimulation chez le Canard domestique. C. R. Acad. Sci. Paris vol. 236, p. 133.

Assenmacher, I. et Benoit, J. - 1953 - Répercussions de la section du tractus porto-tubéral hypophysaire sur la gonadostimulation par la lumière chez le Canard domestique. C. R. Acad. Sci. Paris, vol. 236, p. 2002.

Benoit, J. et Assenmacher, I. - 1951 -

Arch. Anat. microsc. et Morph. expér. vol. 40, p. 27.

Benoit, J. et Assenmacher, I. - 1951 - Circulation porte tubéro-préhypophysaire chez le canard domestique. C. R. Soc. Biol. Paris Vol. 145, p. 1112.

Benoit, J. et Assenmacher, I. - 1951 - Dispositifs nerveux de l'éminence médiane: leurs rapports avec la vascularisation hypophysaire chez le canard domestique. C. R. Soc. Biol. Paris, vol. 145, p. 1395.

Benoit, J. et Assenmacher, I. - 1951 - Contribution à l'étude des relations hypothalamo-hypophysaires et de leur rôle dans la gonadostimulation chez le canard domestique. J. de Physiol. Paris, vol. 43, p. 643.

Benoit, J. et Assenmacher, I. - 1952 - Influences des lésions hautes et basses de l'infundibulum sur la gonadostimulation chez le Canard domestique. C. R. Acad. Sci. Paris, vol. 235, p. 1547.

No.

Bertil Hanström

(Zoological Institute, University of Lund, Sweden).

The neurohypophysis in the series of mammals.

embryological
Numerous ~~embryonic~~ investigations show that the mammalian neurohypophysis is formed by proliferation of the saccus infundibuli of the hypothalamus, though detailed information is scarce. The morphology and histology of its two principal regions, the eminentia mediana and the processus infundibuli, show a progressive evolution, the first stages of which occur in the lowest order of mammals, the egg-laying Monotremata. In the mammalian series three histological stages in the development of the eminentia mediana can be distinguished:

1. In the Monotremata the eminentia consists of an internal, ependymal, fibrous layer with numerous nuclei, and an external layer of delicate nerve endings with no or few nuclei. The outer surface, which is completely flat, is covered by a dense capillary net, but there are no capillaries within the nervous tissue except very few in the peripheral regions of the organ;

2. In the majority of mammalian orders and species the histology of the eminentia is in principle similar to that of the Monotremata, though the number of nuclei, which have migrated from the internal ependymal into the middle and external regions, may be considerable. An important difference is, however, the presence of a particularly rich vascularization of the external layer consisting of capillary loops of the portal system. In addition, the external surface of the

eminentia is very often corrugated, which means an enlarged contact between the external layer and the blood vessels;

3. In several species of the orders Hyracoidea, Artiodactyla and Primates the continuous external layer is gradually reduced, and perivascular layers of the same structure are formed around the blood vessels, which extend further inwards, while the border between the original internal and external layers becomes obliterated.

Considering morphological as well as histological facts, the following provisional arrangement of the different types of the processus infundibuli in mammals may be proposed:

1. In Monotremata (type of the Tasmanian ant-eater), a more or less sac-like process (surrounding the spacious recessus hypophyseus), the walls of which are lobulated by connective tissue septa containing the blood vessels. The tractus supraoptico-hypophyseus branches, joins the lobules, and terminates in layers of delicate nerve endings adhering to the peripheral walls of the lobules.

2. In Monotremata (the platypus) and Marsupialia (American opossums and Trichosurus), an almost solid or completely solid process, which is typically lobulated by septa in which the blood vessels are situated and in which the layers of fine nerve endings lie at the outer surface of the lobules;

3. In Marsupialia (type of the short-tailed wallaby), an almost solid process, in which the periphery is lobulated as in group two, but in which the central regions are richly vascularised as in higher mammals and the blood vessels are surrounded by perivascular layers of delicate nerve endings;

4. In certain Eutheria of the order Carnivora (the genett, the bears, and the feline mammals), a more or less sac-like process as in group one, the thicker walls of which, in spite of the morphological similarity, are not lobulated, but show the same alternation of regions of delicate perivascular nerve endings and coarser fibre bundles as in the centre of the process of the wallaby and in the whole process of the next group;

5. In the majority of the Eutheria, an almost solid or completely solid process which has the same histological structure as in group four.

This brief outline of the evolution of the neural lobe in the mammalian series shows that the chief subdivisions of the lobe, the eminentia mediana and the processus infundibuli, in respect to their vascularisation and histology, have been transformed in a very similar way. Thus in both of these structures cells migrate from the ependyma of the recessus hypophyseus in a centrifugal direction, producing the pituicytes, while connective tissue and blood vessels from the surface of the neurohypophysis migrate into the walls in a centripetal direction. During these processes, in several groups of mammals two opposite currents of tissue elements have passed the original border between the internal and external layers of the eminentia and thus have obliterated it. As a result bundles of axons from the hypothalamic nuclei together with pituicytes, blood vessels and connective tissue, build up the specific architecture of the neural lobe in higher mammals.

The terminal stage in the histological evolution of the eminentia mediana is thus reached in some Hyracoidea, Artiodactyla and Primates,

in which the outcome of the evolution is an organ, whose structure strikingly reminds one of that of the processus infundibuli in the great majority of Eutheria. After the initial stages of a higher differentiation have been passed already in the Marsupialia, the evolution of the eminentia has been very slow and reached the same level of differentiation as that of the processus infundibuli of higher mammals only in the three orders mentioned, and in these only in a small number of species. The processus infundibuli may often become still more complicated by the immigration of elements from the pars intermedia. Such elements are groups of intermedia cells, mucous gland-cells, tubular glands, and lymphoid tissue.

The functional importance of the changes described is evidently a considerable increase of the contact surface between the nerve endings of the eminentia mediana and the processus infundibuli on the one hand, and the blood vessels on the other. According to Wingstrand the blood of the capillaries in the eminentia of birds is separated from the glandular layer only by the endothelium of the vessels, by one thin reticular membrane, and perhaps by a continuous glia sheath. The conditions for an exchange of substances between the blood and the layers of perivascular nerve endings are, therefore, especially favorable. In the processus infundibuli the neurosecretory substance which is stainable with Gomori's chrome hematoxylin has actually been observed to enter the blood vessels. The histological evolution of the infundibular process of mammals thus means a continuously increased promotion of the delivery of hormones connected with the neurosecretory substance into the blood. Since the same histological transformation is observed in the eminentia

mediana in which likewise a colloid, though in our observations not stainable with Gomori's chrome-hematoxylin, has been found, this appears to be a strong argument in favor of the neurosecretory function of the nuclei tuberis, the nerve fibres of which constitute the external layer of the eminentia. Together with important arguments deduced from experimental investigations the consequences of this conclusion for the interpretation of the connections between the hypothalamus and the pars distalis of the glandular lobe of the hypophysis by means of the portal vessels are obvious.

The complete text of this paper will appear in Zeitschrift für Zellforschung, Bd. 37, 1953.

No.

Karl Georg Wingstrand

(Zoological Institute, University of Lund, Sweden)

The ontogeny of the neurosecretory system in chick embryos.

The substances in the supraoptic nucleus, the neurohypophysis, and the subcommissural organ, which stain blue in sections treated with Gomori's chrome-hematoxylin-phloxin method, were studied in embryos of white leghorn chicks which were incubated at 39°C and fixed at different ages with Bouin-Allen. In parallel experiments the embryos were tested for posterior lobe hormones in the subcommissural organ and in the hypothalamic-hypophysial region. Toads (Bufo bufo) were used as test animals. Since the water balance of toads is influenced mainly by the antidiuretic fraction of posterior lobe extracts, there is reason to believe that the hormone tested for is the antidiuretic hormone.

The neurosecretory substance becomes stainable with chrome-hematoxylin in the neurohypophysis and in the supraoptic nucleus of chick embryos at the same time, viz. on the 13th or 14th day of incubation. This observation is in agreement with the idea of a transport of the substance from the supraoptic nucleus to the neurohypophysis - but it is of course no positive proof.

The neurosecretory substance appears in the nerve cells of the supraoptic nucleus before the Nissl bodies have been formed. Thus visible Nissl bodies are not necessary for the synthesis of the neurosecretory material, but there may be present, of course, homogeneously dispersed Nissl substance in the cells already before synthesis of the secretory product begins. Such diffuse Nissl substance cannot be discovered in sections stained with chrome-hematoxylin studied with the light microscope.

At the time of the first appearance of neurosecretory substance in the supraoptic nerve cells and in the neurohypophysis the substance looks homogeneous, or the granules are on the borderline of visibility. Larger distinct granules are formed later both in the cells of the nucleus supraopticus and in the neurohypophysis. They are, therefore, probably secondary aggregates and may indicate that material is being stored. "Herring bodies" of typical appearance are not present in the neurohypophysis until after hatching, and can therefore not be an essential part in the secretory mechanism, but are probably secondary aggregations or sometimes perhaps swollen nerve endings. These findings do not support the interpretation of the neurosecretory phenomena given by Hagen (1952), who believes that cells of the supraoptic nucleus migrate to the neurohypophysis and become loaded with neurosecretory substance on the way. The disintegration of such colloid-loaded cells (Herring bodies according to Hagen) and perhaps also the disintegration of nerve fibers is supposed to give rise to the granular neurosecretory material in the neurohypophysis. However, in the chick embryo there are no Herring bodies which could be interpreted as migrating cells nor are there any degenerating cells in the supraoptic nucleus before hatching, but there are neurosecretory granules in both the nucleus supraopticus and the neurohypophysis.

Posterior lobe hormone is present in the hypothalamic region of the chick embryo already on the 9th or 10th day of incubation whereas the neurosecretory substance becomes visible on the 13th or 14th day. This discrepancy could be explained by assuming that the hormone and the neurosecretory material are not necessarily the same, or that the histological staining method and the pharmacological testing method are not equally sensitive.

The substance which is secreted by the subcommissural organ from the 6th day on and which also stains blue with chrome-hematoxylin, has no effect on the water balance of the toad and is therefore - in contrast to the neurosecretory substance of the hypothalamic-hypophysial system - not associated with posterior lobe hormones. It contributes probably to the formation of Reissner's fibre.

Finally it ought to be pointed out that in embryos incubated for 10 to 12 days an antidiuretic effect is obtained from the hypothalamic-hypophysial region, in which as yet no granules stainable with chrome-hematoxylin can be seen. By contrast, no such hormonal effect is obtained from the subcommissural organ which contains considerable amounts of material staining with Gomori's method. These two observations show that it is not justified to use the method of Gomori as a specific test for antidiuretic hormone or for posterior lobe hormones in general. The relationship between the stainable substance and the hormones which most probably exists, must be rather complicated.

The complete test of this paper will appear in:

Wingstrand, K. G. —1953 - Neurosecretion and antidiuretic activity in chick embryos with remarks on the subcommissural organ. Arkiv för Zoologi (Uppsala, Sweden)

No.

Ernst Scharrer

(Department of Anatomy, University of Colorado, Denver, Colorado, USA)

Neurosecretion in the vertebrates: a survey.

The concept of neurosecretion developed from the observation of nerve cells which also show the cytological characteristics of gland cells. Neurosecretory cells are neurons with Nissl bodies, dendrites, axons and neurofibrils. They produce granules and droplets of substances which can be stained by a variety of methods of which the chrome-hematoxylin-phloxin method of Gomori has been shown by Bargmann to be particularly suitable. The neurosecretory cell can be recognized, therefore, by its cytological characteristics. There are, in addition to these basic properties, a number of features which are observed in some species of animals, but not in others. As an example, the occurrence of nuclei with peculiar shapes indicating their participation in the elaboration of the secretory product may be mentioned. Neurosecretory cells are not distributed at random, but form conspicuous and well defined groups within the central nervous system. In the vertebrates, groups of neurosecretory cells are distinguished from other aggregations of cells in the central nervous system not only by their cytological characteristics, but also by their extraordinarily rich blood supply, and by the fact that they are usually densely packed and as a group sharply marked off from the surrounding nervous tissue.

Throughout the vertebrates, neurosecretory cell groups are found in the hypothalamus. In the cyclostomes, selachians, teleosts, and amphibians the large cells of the nucleus praeopticus secrete stainable material. In the reptiles, birds, and mammals there are two nuclei, the nucleus supraopticus

and nucleus paraventricularis, which contain cell types similar to those constituting the preoptic nucleus of the lower vertebrates. The material produced by the neurosecretory cells of the vertebrate hypothalamus is characteristically and selectively stainable with chrome-hematoxylin. In all cases, the material can be traced along the axons into the posterior lobe of the pituitary where it is stored in the nerve endings.

In the vertebrates, there are other, less well known groups of neurosecretory cells. In teleosts, although not in all species, the cells of the nucleus lateralis tuberis secrete a material which does not stain with chrome-hematoxylin. The activity of these cells appears to be seasonal, i.e. it is evident in summer and subsides in winter. Another group of secreting nerve cells has been found in the tegmentum of the midbrain of fishes and amphibians. Its significance is unknown. Cells with large, irregularly shaped nuclei occur in the spinal cord of skates; the material secreted by these cells does not stain with chrome-hematoxylin. The nucleus of the nervus terminalis in teleost fishes should be included among the neurosecretory cell groups, but very little is known about it. Also, peripherally located autonomic ganglia contain secreting nerve cells, but no systematic study has been undertaken to survey the occurrence of neurosecretory cells outside the central nervous system.

A question of considerable interest concerns the role of the different cellular constituents in the elaboration of the secretory granules. There is some evidence that the basophil constituents of the neurosecretory cells, i.e. the Nissl substance, the basophil cytoplasm, and the nuclear chromatin may be used up in the process of the elaboration of the neurosecretory substance. No direct relationship has been observed between the emergence

within the cells of the smallest granules and the Golgi material or the mitochondria. However, it is only too well known how difficult it is to determine the antecedents of secretory granules. In fact, most statements based on observations with the light microscope are meaningless, since the granules are formed at the submicroscopic level. The only observation of significance concerns the increase of the neurosecretory material in the cell and the concurrent decrease in the basophil material. This may mean that the neurosecretory material is elaborated at the expense of the basophil substances, but the matter requires further careful investigation before a more definitive statement can be made.

Apart from the cytological processes taking place in the elaboration of the neurosecretory material, the study of the finer structure of secreting nerve cells is of great interest and there is still a large field to be explored. The question arises, for instance: what happens to the neurofibrils in cells which become all but filled with secretory granules? In many instances the nuclei of neurosecretory cells look very different from what one is used to see in nerve cells. Often the nuclei show various lobes and subdivisions, invaginations and processes. Similar nuclear shapes have been described in various types of gland cells and have been interpreted to indicate a need for an increase of the nuclear surface. The same interpretation is probably applicable to neurosecretory cells.

Little is as yet known about the cytochemistry of neurosecretory cells. In mammals, such cells have a high content of acid phosphatase, but in this the neurosecretory cells are not unique. Other nerve cells, as for instance the cells of the mesencephalic V nucleus of certain vertebrates, are also distinguished by the presence of large amounts of acid phosphatase in the

cytoplasm. Of particular interest is, of course, the chemical nature of the secretory granules. In the mammalian hypothalamus, they contain the active polypeptides of the posterior lobe hormones and a stainable component which is soluble in organic solvents.

Whereas in the invertebrates evidence that the neurosecretory cells produce hormones began to accumulate soon after these cells had been first described, no corresponding proof of their endocrine nature in the vertebrates could be offered for some time. Only since Bargmann and his coworkers had shown that the neurosecretory material in the hypothalamo-hypophyseal system contains the hormones whose origin had been ascribed to the posterior lobe, was the endocrine function of the neurosecretory cells of the hypothalamus established. As a result, the posterior lobe of the pituitary can no longer be considered as an endocrine organ in a strict sense. Its function is that of an organ of storage for the hormones produced by cells in the hypothalamus. The hypothalamo-neurohypophyseal system is the only neurosecretory system in the vertebrates whose functional significance is now known. Still unexplained is the role of the other neurosecretory centers enumerated above.

The extensive literature on which this survey was based, is quoted in the following reviews:

Scharrer, E. and B. Scharrer – 1937 – Über Drüsen-Nervenzellen und neurosekretorische Organe bei Wirbellosen und Wirbeltieren. Biol. Reviews, Vol. 12, p. 185.

Scharrer, E. and B. Scharrer – 1940 – Secretory cells within the hypothalamus. Res. Publ. Ass. nerv. ment. Dis., Vol. 20, p. 170.

Scharrer, E. and B. Scharrer – 1945 – Neurosecretion. Physiol. Reviews, Vol. 25, p. 171.

Scharrer, E. and B. Scharrer – 1954 – Neurosekretion, in Handbuch der mikrosk. Anat. d. Menschen, Herausg. von Möllendorff und Bargmann. Springer (in press).

No.

W. Bargmann

(Anatomisches Institut der Universität Kiel, Deutschland)

Die endokrine Funktion neurosekretorischer Zellen bei Wirbeltieren.

Das Referat von Ernst Scharrer enthält so viele Hinweise auf die endokrine Funktion neurosekretorischer Zellen bei den Vertebraten, dass ich mich hier auf einige ergänzende Bemerkungen beschränken kann, zumal eine Reihe auch referierender Veröffentlichungen bereits vorliegt (Bargmann und Scharrer, 1951; Bargmann, 1953).

Zunächst sei die Frage angeschnitten, ob die im vegetativen Nervensystem sich abspielenden, histologisch fassbaren Sekretionsvorgänge nur dessen zentralem Anteil vorbehalten sind, oder ob neurosekretorische Prozesse auch im peripheren vegetativen System ablaufen. Verschiedene Befunde sprechen meines Erachtens für das Vorkommen von Stoffbildungsvorgängen auch in peripheren vegetativen Ganglienzellen. Es handelt sich einmal um das Auftreten von Kolloidtröpfchen im Cytoplasma sympathischer Nervenzellen von Säugern (Lennette und Scharrer, 1946; Eichner, 1951, 1952), ferner um das regelmässige Vorkommen grosser, aus dünnflüssigem Sekret bestehender Tropfen in Ganflienzellen des Nebennierenmarkes (Eichner, 1952). Die kolloidhaltigen Ganglienzellen weisen keine degenerativen Veränderungen auf, ebensowenig wie die vakuolisierten, durch multiple Flüssigkeitsblasen teilweise stark aufgetriebenen Nervenzellen des Grenzstranges. Eine signifikante Zunahme der Zahl solcher Zellen beobachteten

Lehmann und Stange (1953) im Ganglion cervicale uteri der Ratte während der Gravidität. Es bedarf experimenteller Untersuchungen, um die funktionelle Bedeutung dieser Phänomene aufzuklären. Wir wissen bisher nicht, ob die erwähnte intrazelluläre Stoffbildung tatsächlich etwas mit der Produktion von Hormonen zu tun hat.

Dagegen hat sich zeigen lassen, dass die neurosekretorische Tätigkeit bestimmter zentraler Abschnitte des vegetativen Systems, nämlich der diencephalen Kerne Nucleus supraopticus und paraventricularis der Säugetiere der Bildung von Hormonen dient. Es handelt sich um jene Zwischenhirnkerne, deren Neurosekret ebenso wie das des Nucleus praeopticus der Fische und Amphibien mit Gomori's Chromhämatoxylin-Phloxinfärbung elektiv dargestellt werden kann (Bargmann, 1949). Wie bekannt, entsteht im Cytoplasma der Gan lienzellen der genannten Kerne ein granuläres bzw. tropfiges Sekret, in manchen Fällen anscheinend auf Kosten der Nisslsubstanz.Das anfärbbare Material wurde von mir ursprünglich kurz "gomoripositiv" genannt. Diese Bezeichnung ist jedoch schon im Hinblick auf die Fülle der von Gomori angegebenen Methoden für weiteren Gebrauch nicht mehr zu empfehlen. Ich möchte unterstreichen, dass die Färbung des Neurosekrets mit Chromhämatoxylin-Phloxin von mir nicht als spezifisch im Sinne einer chemischen Reaktion aufgefasst wurde (vgl. hierzu Goslar, 1953).Schiebler (1951, 1952) hat gezeigt, dass es bei geeigneter Vorbehandlung gelingt, das Neurosekret mit verschiedenen basischen Farbstoffen hervorzuheben. Die Chromhämatoxylin-Methode hat sich freilich als die brauchbarste zur Darstellung

des Neurosekrets erwiesen und auch für die Erforschung der Neurosekretion bei Wirbellosen wertvolle Dienste geleistet.

Das im Perikaryon entstandene Neurosekret wird entlang den Nervenzellfortsätzen bzw. innerhalb der Oberfläche der Fortsätze bis in die Neurohypophyse transportiert und dort gestapelt. Die Berechtigung der Transporthypothese geht aus den Versuchen von Hild (1951), Stutinsky (1951), Scharrer und Wittenstein (1952), sowie Hild und Zetler (1953) hervor, sodass ins Einzelne gehende Erörterungen sich erübrigen. Summarisch sei auf das Auftreten einer Neurosekretanreicherung in den proximalen Stümpfen der Fasern der "neurosekretorischen Bahn" (Tractus praeoptico- bzw. supraoptico-hypophyseus) nach Durchschneidung des Hypophysenstieles hingewiesen. Im weiteren Verlauf des Symposiums wird auf die Transportfrage eingegangen werden, die auch für die Beurteilung der neurosekretorischen Systeme von Invertebraten eine wichtige Rolle spielt.

Wenn man die Frage nach der Funktion der neurosekretorischen Bahn stellt, die Hypothalamus und Neurohypophyse miteinander verbindet, so hat man zunächst die Tatsache zu berücksichtigen, dass in den verschiedenen Abschnitten dieser Bahn Veränderungen dann auftreten, wenn Belastungen des Wasserhaushaltes vorliegen. Beispielsweise kommt es zu einer Verarmung der Neurohypophyse des Frosches an färberisch darstellbarem Neurosekret, wenn die Tiere in trockenem Milieu leben (Hild, 1951). Das gleiche Phänomen kann man bei der Ratte im Falle einer gesteigerten Diurese nachweisen (Kratsch, 1951). Auch an den Kernen der Ganglienzellen der neurosekretorischen Zwischenhirnkerne treten beim Dursten Veränderungen auf, die sich u.a. in einer

signifikanten Vergrösserung der Zellkerne (Eichner, 1952; Macher, 1952) und einer Grössenzunahme der Nucleo$\not{p}$en (Hillarp, 1949; Ortmann, 1951) bemerkbar machen. Diese Erscheinungen fassen wir als Ausdruck der Aktivitätssteigerung der neurosekretorischen Neurone auf. Unter extremen Bedingungen des Durstes werden auch degenerative Veränderungen der Ganglienzellen beobachtet.

Nach diesem Hinweis liegt die Vermutung nahe die färbbare Komponente des Neurosekrets könne die Trägersubstanz eines den Wasserhaushalt steigernden Hormons verkörpern, nämlich des Adiuretins der Neurohypophyse. Diese Vermutung konnte durch eine Reihe experimenteller Untersuchungen von Hild und Zetler (1951-53) bestätigt werden. Das Neurosekret enthält fernerhin Oxytocin und Vasopressin. Extrakte aus den isolierten Nuclei supraoptici und paraventriculares, dem Tuber cinereum als der Durchgangsstelle der neurosekretorischen Bahn und dem Hinterlappen als ihrer Endstation enthalten die drei Wirkstoffe in gut definierbaren Mengen (Hund). Der Hormonnachweis erfolgte mit den in der Pharmakologie üblichen Methoden am Blasenfistelhund (Adiuretin), am isolierten virginellen Meerschweinchenuterus (Oxytocin) und am Blutdruck der dekapitierten Katze (Vasopressin). Es ergab sich weiterhin, dass das Kerngebiet einen geringeren Hormongehalt als das Tuber und dieses als der Hinterlappen aufweist. Extrakte angrenzender Hirnpartien erwiesen sich als wirkungslos. Interessanterweise konnte auch aus der bei Stieldurchtrennung zu beobachtenden Neurosekretanreicherung im proximalen Stumpf eine höhere Hormonausbeute gewonnen werden (Hund), als als man sie unter normalen Umständen an der

entsprechenden Stelle findet (Hild und Zetler, 1953), während der distale abgetrennte Bahnabschnitt an Neurosekret und Hormonen verarmt. Mit diesen Befunden gewinnt die Transporthypophese weiterhin an Wahrscheinlichkeit.

Aus den Untersuchungen des neurosekretorischen Zwischenhirn-Hypophysensystems ergibt sich eine neue, die Bewertung der Neurohypophyse betreffende Konzeption (Bargmann, 1951; Bargmann und Scharrer, 1951): Der Hinterlappen stellt das Stapel- und Abgabeorgan der in den Zwischenhirnkernen gebildeten, an eine elektivefärbbare Trägersubstanz gebundenen Hormone dar. Die Ganglienzellen der Kerne und nicht die Pituicyten des Hinterlappens sind die Hormonbildner. Ob diesen Gliazellen bei der Freisetzung der Hormone aus den Nervengeflechten des Hinterlappens und der Abgabe der Wirkstoffe an die Blutbahn eine Rolle zukommt, bedarf der Untersuchung. In einigen Fällen berichten Forscher über das Auftreten von Mitosen der Pituicyten bei Belastung des Wasserhaushaltes; in anderen Fällen wurden Zellteilungen vermisst.

Mit der Frage der Natur der Trägersubstanz befassen sich Untersuchungen von Schiebler (1951, 1952), denen zufolge die mit Chromhämatoxylin färbbare Komponente des Neurosekrets wahrscheinlich einen Glykolipoproteinkomplex darstellt.

Die Feststellung der endokrinen Tätigkeit der Ganglienzellen des Nucleus supraopticus und paraventricularis hat eine Reihe neuer Fragen aufgeworfen, aus denen ich hier nur die nach der Einreihung des neurosekretorischen Systems in die Kette der übrigen innersekretorischen Drüsen herausgreife. Da auch die Nebennieren an der Steuerung des Wasser- und Salzhaushaltes beteiligt sind, hat sich Eichner (1953) mit den Beziehungen

zwischen Nebennierenrinde und Zwischenhirn-Hypophysensystem beschäftigt. Nach Eichner bewirkt gesteigerte Na-Zufuhr bei der Ratte eine Aktivierung des Systems, die sich in Abnahme des Neurosekrets sowie Kern- und Nucleolonvergrösserung in den Nuclei supraoptici und paraventriculares äussert. In der Nebennierenrinde treten Merkmale einer Tätigkeitsminderung der Zona glomerulosa auf, während die Kernvergrösserung in der Zona fasciculata für Tätigkeitssteigerung spricht. Verminderte Na-Zufuhr ist nicht von Zeichen einer Aktivitätssteigerung im Zwischenhirn-Hypophysensystem gefolgt. Die Zona glomerulosa erscheint aktiviert, die Zona fasciculata als in Ruhe befindlich. Nach Adrenalektomie kommt es zur Verminderung des Neurosekrets im Hinterlappen und einer geringen Aktivierung der Zwischenhirnkerne. Eichner ist der Ansicht, dass das neurosekretorische Zwischenhirnsystem und die Zona glomerulosa der Nebenniere zueinander in einem antagonistischen Verhältnis in Bezug auf den Na-Haushalt stehen.

Während wir nunmehr über Einblicke in die endokrine Funktion des neurosekretorischen Zwischenhirn-Neurohypophysen-Systems verfügen, ist uns die Bedeutung anderer neurosekretorischer Elemente des Zentralnervensystems noch verborgen. Ein weites reizvolles Feld der Forschung liegt hier brach.

Bargmann, W. - 1949 - Über die neurosekretorische Verknüpfung von Hypothalamus und Neurohypophyse. Z. Zellforsch. Vol. 34, p.610.

Bargmann, W. - 1951 - Zwischenhirn und Neurohypophyse. Med. Mschr. H. 7, p. 466.

Bargmann, W. - 1953 - Zwischenhirn und Hypophyse. 1.Hauptbericht Tg. Dtsch. Ges. f. Gynäkologie München 1952. 29. Ber. (im Druck).

Bargmann, W. - 1953 - Zwischenhirn-Hypophysen-System, Neurosekretion und Nebenniere. Geburtshilfe und Frauenheilk. Vol. 13, p. 193.

Bargmann, W. - 1953 - Über das Zwischenhirn-Hypophysensystem von Fischen. Z. Zellforsch. Vol. 38, p. 275.

Bargmann, W. und Hild, W. - 1949 - Über die Morphologie der neurosekretorischen Verknüpfung von Hypothalamus und Neurohypophyse. Acta Anatomica. Vol. 8, p. 264.

Bargmann, W., Hild, W., Ortmann, R. und Schiebler, Th. H. - 1950 - Morphologische und experimentelle Untersuchungen über das hypothalamisch-hypophysäre System. Acta neurovegetativa. Vol. 1, p. 233.

Bargmann, W. und Scharrer, E. - 1951 - The site of origin of the hormones of the posterior pituitary. Am. Scientists Vol. 39, p. 255.

Bargmann, W. und E. Jacob, K. - 1952 - Über Neurosekretion im Zwischenhirn der Vögel. Z. Zellforsch. Vol. 36, p. 556.

No.

Valdo Mazzi

(Istituto di Anatomia Comparata dell' Universita di Firenze, Italia)

I fenomeni neurosecretori nella femmina del Tritone crestato in condizioni sperimentali.

L'Autore ha studiato il sistema ipotalamo-ipofisario della femmina del Tritone crestato normale e nelle seguenti condizioni sperimentali: lesioni ipotalamiche, ipofisectomia, carico osmotico, allevamento in ambiente secco, trattamento con acetato di rame. Negli animali ipotalamo-lesi in estate o allevati a temperatura artificiale di tipo estivo, l'Autore ha osservato in un primo tempo un aumento dell'attività secretoria nelle cellule del nucleo magnocellulare preottico, che poi declina per esaurirsi completamente 50 giorni circa dopo l'intervento operatorio, senza che ne segua atrofia cellulare. Il neurosecreto si accumula nel moncone prossimale del fascicolo preottico-ipofisario, le fibre lentamente degenerano in direzione petale. Nella neuroipofisi la atrofia tissulare e la rarefazione di sostanza neurosecretoria , già apprezzabile dopo 9 giorni, appaiono estremamente spinte dopo 50 giorni circa. L'atrofia

della neuroipofisi, la deplezione in essa del neurosecreto, e il calo dell'attività secretoria nelle cellule del nucleo magnocellulare preottico sono meno accentuate negli animali, allevati, d'inverno, a temperatura naturale.

Negli animali ipofisectomizzati l'Autore ha osservato che durante la prima settimana l'attività secretoria nelle cellule del nucleo magnocellulare preottico tende ad aumentare. Dal 12° giorno in poi lentamente declina, ma non si esaurisce del tutto neppure dopo 36 giorni, ed entro questo termine le cellule secernenti non degenerano. La quantità di neurosecreto entro le fibre del fascicolo preottico-ipofisario è scarsa nei primi tre giorni, aumenta e si mantiene rilevante dal 5° al 12° giorno, e progressivamente poi diminuisce, senza mai scomparire del tutto. In corrispondenza del tuber le fibre del fascicolo preottico-ipofisario degenerano, e in esse si accumula sostanza neurosecretoria. La degenerazione progredisce lentamente in direzione petale.

Il carico osmotico (allevamento in soluzione all'1% di cloruro di sodio) tanto in animali integri che in animali ipotalamo-lesi ha portato, nel corso di 10 giorni, alla degenerazione delle cellule neurosecernenti del nucleo magnocellulare preottico, ad una forte diminuzione del neurosecreto entro gli assoni dell' fascicolo preottico-ipofisario e ad una netta deplezione della colloide neuroipofisaria, non accompagnata da mitosi nei pituiciti.

Del pari l'allevamento in ambiente secco ha determinato solo in un primo tempo un'esaltazione dei fenomeni neurosecretori nelle cellule del nucleo magnocellulare preottico o ad un incremento dei fenomeni di trasporto lungo la "via neurosecretoria"; in un secondo tempo però fatti degenerativi a carico delle cellule secernenti e una più o meno marcata deplezione della colloide nella neuroipofisi.

Infine gli effetti ottenuti con iniezioni di acetato di rame sono troppo poco netti per autorizzare qualsiasi deduzione.

Mazzi, V. I fenomeni neurosecretori nella femmina del Tritone crestato in condizioni sperimentali. Zeitschrift für Zellforschung (in corso di stampa).

No.

Gerhard Zetler & Walther Hild

(Pharmakologisches Institut und Anatomisches Institut
der Universität Kiel, Deutschland).

Über die neurosekretorische Tätigkeit des hypothalamisch-neurohypophysären Systems der Säugetiere.

Scharrer (1928, 1930 und später) fand morphologische Anzeichen für eine sekretorische Tätigkeit in den Ganglienzellen der Nuclei supraoptici und paraventriculares des vorderen Hypothalamus. Bargmann (1949, 1950) gelang es, mit Hilfe von Gomoris Chromalaun-Hämatoxylin-Phloxinfärbung ein einheitliches neurosekretorisches System, bestehend aus den erwähnten hypothalamischen Kerngebieten, dem Tractus supraoptico-hypophyseus und seinen Endigungen in der Neurohypophyse, darzustellen; dabei färbt sich ein granuläres, bzw. tropfiges intrazelluläres Sekretionsprodukt. Nachdem wir (Hild und Zetler, 1951 a, b) gezeigt hatten, dass in allen Teilen dieses Systems Adiuretin, Vasopressin und Oxytocin gefunden werden können, verglichen wir die Verhältnisse bei Hund, Rind, Schwein und Mensch miteinander (Hild und Zetler, 1952 a, b).

Dabei fiel uns eine sehr enge quantitative Parallelität zwischen der Konzentration an histologisch darstellbarem Neurosekret einerseits und an Hormonen andererseits auf; dies gilt nicht nur für die Unterschiede zwischen den

einzelnen Species, sondern auch für individuelle Schwankungen. Die höchsten Konzentrationen an sichtbarem Neurosekret und Hormonen fanden wir beim Hund, die niedrigsten beim Menschen. Das histologisch darstellbare Neurosekret ist jedoch nicht identisch mit den Hormonen oder mit einem dieser Wirkstoffe, etwa dem Adiuretin, wie folgende Versuche (Hild und Zetler, 1953 a) beweisen: Durch 12stündiges Einlegen von frischen Schweine-Neurohypophysen in ein Gemisch von 2 Teilen absolutem Äthanol und 1 Teil Chloroform verschwindet im histologischen Bild die mit Gomoris Methode färbbare Komponente des Neurosekrets; diese kann im Äthanol-Chloroform-Gemisch nachgewiesen werden. Der Hormongehalt der so behandelten Neurohypophysen bleibt konstant. Wir glauben, dass es sich bei der färbbaren Komponente des Neurosekrets um eine lipoide Träger- oder Vehikelsubstanz handelt und weisen darauf hin, dass der Begriff "Neurosekret" demnach also einen Komplex aus einem histologisch darstellbaren und einem histologisch nicht darstllbaren Anteil umfasst.

Um zu klären, ob das die Hormone enthaltende Neurosekret entweder in der Neurohypophyse oder im Hypothalamus gebildet ~~wurden~~ wird, unternahmen wir folgende Versuche (Hild und Zetler, 1953 b): Je 10 Schäferhunde dursteten 8 und 14 Tage lang, weitere je 10 Hunde erholten sich nach 14tägigem Dursten 12 Stunden, 1, 4 und 8 Tage lang; bei 10 Hunden wurde vor dem Beginn einer 4tägigen

Erholungsperiode der Tractus supraoptico-hypophyseus auf diasphenoidalem Wege durchschnitten. Bei jedem einzelnen Gehirn wurden die Teile des hypothalamisch-neurohypophysären Systems isoliert und sowohl histologisch (Färbung von Serienschnitten nach Gomori, Erfassung der Neurosekretmenge durch Extinctionsmessung) und pharmakologisch (Quantitative Testung von Adiuretin, Vasopressin und Oxytocin) untersucht. An färbbarem Neurosekret wie an Hormonen verarmte während des Durstens die Neurohypophyse schneller als die hypothalamischen Anteile des Systems; auch während der Erholung eilte die Neurohypophyse voran. Der Nucleus paraventricularis zeigte deutlich geringere Schwankungen im Neurosekret- und Hormongehalt als der Nucleus supraopticus. Nach Durchschneidung des Tractus supraoptico-hypophyseus verminderten sich Hormon- und Neurosekretbestand auch während der Erholungsphase in der Neurohypophyse weiter, während ~~sich~~ sie proximal von der Operationsstelle über den Kontrollwert (4 Tage Erholung nach 14tägigem Dursten ohne Stieldurchtrennung) hinaus zunahmen. Hormone und färbbares Neurosekret stauten sich am stärksten im proximalen Stumpf des Tractus supraoptico-hypophyseus an. Dies dürfte ein ~~kleiner~~ klarer Beweis dafür sein, dass Adiuretin, Vasopressin und Oxytocin sowie das histologisch darstellbare Neurosekret nicht von der Neurohypophyse, sondern von den Nuclei supraoptici und paraventriculares des vorderen Hypothalamus gebildet

werden. Der Tractus supraoptico-hypophyseus ist der Transportweg für diese Produkte der Neurosekretion, die Neurohypophyse nur ein Speicherungs- und Ausscheidungsort.

In den einzelnen Phasen des Experimentes zeigten Adiuretin, Vasopressin und Oxytocin quantitative Schwankungen, die völlig unabhämgig voneinander waren. Dies ilt auch für die Unterschiede zwischen den einzelnen Species und für individuelle Schwankungen innerhalb der Species. Sogar bei jedem einzelnen Gehirn stehen in den verschiedenen Abschnitten des hypothalamisch-neurohypophysären Systems die drei Hormonkomponenten zueinander in unterschiedlichem quantitativem Verhältnis. Dies ist nur schwer mit der Vorstellung zu vereinbaren, Adiuretin, Vasopressin und Oxytocin seien nur die Wirkungskomponenten eines oder höchstens zweier Moleküle (Genaueres bei Zetler, 1953). Schliesslich ergab sich, dass die Menge des im histologischen Bild erscheinenden Neurosekrets nur einen groben Anhaltspunkt für die Hormonmenge darstellt. Die Neurosekretkonzentration ging manchmal mit dem mittleren Hormongehalt, manchmal mit der Menge einer der drei Komponenten Hand in Hand. Für das hypothalamisch-neurohypophysäre System muss deshalb vor zu weitgehenden Schlüssen vom histologischen Bild auf das endokrine Geschehen gewarnt werden.

Hild, W. und Zetler, G. - 1951 a - Über das Vorkommen der drei sogenannten "Hypophysenhinterlappenhormone" Adiuretin, Vasopressin und Oxytocin im Zwischenhirn als wahrscheinlicher Ausdruck einer neurosekretorischen Leistung der Ganglienzellen der Nuclei supraopticus und paraventricularis. Experientia (Basel), Bd. 7, S. 189.

Hild, W. und Zetler, G. - 1951 b - Über das Vorkommen der Hypophysenhinterlappenhormone im Zwischenhirn. Arch. exper. Path. u. Pharmakol., Bd. 213, S. 139.

Hild, W. und Zetler, G. - 1952 a - Vergleichende Untersuchungen über das Vorkommen der Hypophysenhinterlappenhormone im Zwischenhirn einiger Säugetiere. Dtsch. Zeitschr. Nervenheilk., Bd. 167, S. 205.

Hild, W. und Zetler, G. - 1952 b - Neurosekretion und Hormonvorkommen im Zwischenhirn des Menschen. Klin. Wschr., Jg. 30, S. 433.

Hild, W. und Zetler, G. - 1953 a - Über die Funktion des Neurosekrets im Zwischenhirn-Hypophysensystem als Trägersubstanz für Vasopressin, Adiuretin und Oxytocin. Zeitschr. ges. exper. Med. Bd. 120, S. 236.

Hild, W. und Zetler, G. - 1953 b - Experimenteller Beweis für die Entstehung der sog. Hypophysenhinterlappenwirkstoffe im Hypothalamus. Pflüger's Arch. ges. Physiol., Bd. 257, S. 169.

Zetler, G. - 1953 - Arch. exper. Path. u. Pharmakol. (im Druck).

No.

F. Stutinsky

(Faculté des Sciences, Paris)

La neurosécrétion chez l'Anguille normale et hypophysectomisée.

La neurosécrétion chez l'Anguille (Anguilla vulgaris) est particulièrement nette et fournit des images très suggestives sur certains points discutés de ce problème.

I - Le noyau de la cellule nerveuse du nucleus magnocellularis preopticus participe au processus neurosécrétoire. C'est là, semble-t-il, un des caractères morphologiques fondamentaux de la cellule neurosécrétrice.

II - L'hydrencephalocrinie est ici évidente: certaines cellules nerveuses du noyau préoptique, de type bipolaire, insinuent un de leurs pôles entre les cellules épendymaires, qui passe dans la lumière du troisième ventricule. La partie cytoplasmique intra-ventriculaire s'élargit, contient des vacuoles et donne parfois l'impression de se dissoudre dans le liquide céphalo-rachidien. Ces images ne représentent qu'un cas particulier d'un phénomène général; cependant le passage du neurosécrétat se produit le plus souvent chez d'autres Vertébrés au niveau de l'ependyme du plancher du 3ème ventricule non pas à partir des cellules nerveuses, mais à partir des fibres nerveuses hypothalamo-neurohypophysaires.

La neurosécrétion possède donc au moins deux voies d'élimination, le troisième ventricule et la neurohypophyse. Il est possible que certaines conditions puissent faire prévaloir l'un ou l'autre mode d'excrétion et engendrer de ce fait des conséquences

physiologiques dont le déterminisme est encore obscur.

III - L'organe sous-commissural et la fibre de Reissner se colorent - comme les cellules neurosécrétrices - électivement par l'hématoxyline de Gomori et par la méthode de Hotchkiss-McManus. La signification de ces structures est discutée et la solution de ce problème demande de nouvelles recherches.

IV - La structure de la fibre neurosécrétrice est hétérogène et certains aspects - dans la mesure où l'on peut écarter l'idée d'un artefact - suggèrent la possibilité d'une activité sécrétoire locale.

V - La neurohypophyse de l'Anguille est innervée par deux systèmes de fibres dont la nature et l'origine sont différentes. Les digitations rostrales de la neurohypophyse qui pénètrent dans les lobes tubériens et antérieurs reçoivent des fibres qui naissent au niveau des noyaux tubériens. Elles ne se colorent pas par l'hématoxyline chromique. Assez souvent on trouve des cellules nerveuses erratiques de ce noyau à l'intérieur de la neurohypophyse. Les digitations postérieures de la neurohypophyse, en contact avec le lobe intermédiaire contiennent le contingent de fibres neurosécrétrices provenant du noyau pré-optique. Le neurosécrétat peut accompagner quelques fibres <u>à l'intérieur</u> du lobe intermédiaire, mais la plus grosse partie s'accumule à la frontière neuro-intermédiaire autour des plexus neurofibrillaires.

VI - L'hypophysectomie chez l'Anguille ne supprime pas la substance colorable par l'hématoxyline de Gomori dans l'hypothalamus. Comme chez la Grenouille, le Rat et le Chien, la substance neuro-sécrétée s'accumule au niveau de la lésion où les fibres sont hypertrophiées, déformées et plus chromophiles. Le noyau pré-optique possède lui aussi plus d'affinité pour l'hématoxyline chromique.

L'origine hypothalamique de la substance colorable par l'hématoxyline chromique est ainsi établie egalement pour les Téléostéens.

Travaux de l'auteur à consulter:

Stutinsky, F. - 1950 - Sur la signification des "corps de Herring" de la neurohypophyse. C. R. Ass. Anat., 37e Réunion, Louvain, p. 493 .

______ 1950 - Colloïde, corps de Herring et substance Gomori-positive de la neurohypophyse. C. R. Soc. Biol., Tome 144, p. 1357.

______ 1951 - Sur l'origine de la substance Gomori-positive du complexe hypothalamo-hypophysaire. C. R. Soc. Biol., Tome 145, p. 367.

______ 1952 - Modalités de la neurosécrétion dans le noyau pré-optique de l'Anguille. Bull. Soc. Zool., Tome 77, p. 240.

No.

Giacomo Azzali

(Istituto di Anatomia Umana dell' Università di Parma, Italia).

Ricerche sulla neurosecrezione negli animali ibernanti.

Nelle ricerche sugli animali ibernanti, dopo le note del 1951 e 1952 in cui era stata vista una forte quantità di sostanza colorabile con l'ematossilina cromica secondo Gomori nei nuclei sopraottico e paraventricolare dei Chirotteri nei mesi invernali e la quasi totale assenza di tale sostanza nel periodo estivo, ho creduto opportuno allargare le indagini sia studiando animali con un letargo diverso da quello dei Chirotteri sia mettendomi in condizioni di esperimentazioni il più severe possibili onde evitare eventuali modificazioni causate dal diverso habitat o da disturbi del mantenimento del letargo stesso. Al fine di essere nelle condizioni migliori di esperimento, per gli studi sui Chirotteri ho proceduto nel seguente modo:

1) prelievo ed uccisione degli animali nel luogo dell'ibernazione durante tutti i mesi invernali e cattura ed immediata uccisione nel periodo estivo onde evitare eventuali stress.

2) soggiorno degli animali per breve tempo fuori dall'ambiente nel quale vivevano durante il letargo.

3) risveglio brusco con soggiorno in termostato o in ambiente riscaldato.

Per queste ricerche mi sono servito di numerose specie di Chirotteri (Verperugo savi, Vesperugo noctula, Rhinolophus ferrum equinum, Rhinolophus euriali, Plecotus auritus) onde avere un quadro il più completo possibile; per la colorazione ho usato il metodo della cromo-allume-ematossilina-floxina di Gomori.

Dall'esame dei preparati dei Chirotteri sottoposti al 1° esperimento posso affermare che nel periodo letargico i neuroni dei nuclei sopraottico e paraventricolare sono ricchissimi di sostanza colorabile col metodo di Gomori la quale tende a disporsi alla periferia del corpo cellulare e lungo il neurite sotto l'aspetto di finissimi granuli. La regione infundibolare, il peduncolo dell'ipofisi e la neuroipofisi presentano anch'essi un abbondante quantita di sostanza colorabile col metodo di Gomori ad aspetto di piccole e grosse goccie.

Nel periodo estivo i neuroni dei nuclei sopraottico e paraventricolare, la regione infundibolare e la neuroipofisi sono caratterizzati dalla mancanza quasi totale dei granuli colorabili con l'ematossilina cromica.

Questo reperto, che per la prima volta ho ottenuto nell'annata 1951, fu riconfermato con le ricerche di controllo fatte negli anni 1952 e 1953.

La sp#iegazione dell'accumulo di sostanza colorabile col

metodo di Gomori nel periodo letargico nei contri neurovegetativi diencefalici si può intendere solo nel senso di una minor utilizzazione del principio antidiuretico per cui si ha Diencefalo da accumulo.

Nel 2° gruppo di esperimenti, osservando cioè Chirotteri che pur lasciati in letargo fu loro cambiato l'ambiente di vita naturale con sostituzione della luce al buio abituale, ho potuto osservare che la sostanza colorabile col metodo di Gomori nei nuclei sopraottico e paraventricolare,nella regione infundibolare e nella neuroipofisi era diminuita lievemente. Questa diminuizione e da imputare ad uno stress da cambiamento di habitat o alla modificazione del fattore di illuminazione? A me sembra che la causa principale da tener presente sia lo stress da cambiamento di habitat il quale determina un modesto consumo del neurosecreto mentre la luce non può influire che in modo tanto scarso da essere trascurata.

Nel 3° gruppo di esperimenti, cioè risveglio brusco con soggiorno degli animali in termostato o in stanza riscaldata, ho potuto osservare una scomparsa quasi totale dai nuclei neurovegetativi diencefalici della sostanza colorabile col metodo di Gomori dopo 60 ore dal risveglio obbligato.

Questa osservazione si può spiegare con un accentuazione funzionale di tutti i metabolismi e così pure di quello idrico-salino con vivace utilizzazione dell'ormone neurosecreto.

Dopo le ricerche sui Chirotteri ho voluto estendere il mio studio ad animali i quali hanno un letargo particolare, diverso da quello dei Chirotteri; per questo tipo di ~~se~~ ricerche ho scelto il Ghiro.

A conclusione delle mie osservazioni posso affermare che nel Ghiro le cellule nervose dei nuclei sopraottico e paraventricolare rivelano modesta sostanza "Gomori positiva" sia nei mesi letargici che nei mesi estivi. La regione infundibolare ed il peduncolo dell'ipofisi non hanno comportamento dissimile. Anche la neuroipofisi non presenta alcuna modificazione quantitativa rispetto ai mesi dell'anno però è il centro più ricco di neurosecreto, rappresentato dai corpi di Hering e da finissimi granuli.

Dopo questi miei studi posso affermare che le ricerche sull'ibernazione nei Chirotteri ed in Myoxus glis non concordano: nei Chirotteri la sostanza "Gomori positiva" che è scarsissima nell'estate si accumula durante l'inverno; nel Ghiro non subisce modificazioni nelle varie epoche dell'anno. Credo che ciò dipenda dal fatto che il letargo non ha caratteristiche biologiche identiche nei diversi animali ibernanti e varie sono pure le modificazioni che l'ibernazione induce sui metabolismi. Da ciò dipende la diversa richiesta di sostanza colorata secondo il metodo di Gomori all'ematossilina cromica.

Per notizie piu precise vedere:

Azzali, G. - 1952 - Ulteriori contributi al problema della neurosecrezione diencefalica dei Chirotteri. Bol. Soc. ital. Biol. sper., Vol. 28, p. 11.

Azzali, G. - 1953 - Ricerche sulla neurosecrezione ipotalamica nei Chirotteri. Rivista di Biologia, Vol. p.

No.

André Stahl & Raymond Seite

(Laboratoire d'Histologie, Faculté de Médecine, Marseille, France)

Cytologie des cellules nerveuses de l'hypothalamus.

Les auteurs ont recherché si un équipement cytologique particulier correspond aux propriétés neurosécrétoires établies à la suite des recherches de Scharrer et de Bargmann. Dans ce but, ils ont étudié l'appareil de Golgi et le chondriome des cellules nerveuses des noyaux supraoptique et paraventriculaire, chez le Chat, le Cobaye et le Rat. Tous les animaux utilisés étant normaux et adultes, l'appareil de Golgi a été mis en évidence par la méthode de Da Fano, le chondriome par la méthode de Regaud. Les auteurs ont fait les constatations suivantes:

Chez tout les animaux étudiés, le grand développement de la zone de Golgi est remarquable. Elle occupe toute la partie du cytoplasme qui est chromophobe avec les colorants basiques, et qui est comprise entre le noyau, souvent en position excentrique, et la couronne périphérique des corps de Nissl. Étudié par la méthode de Da Fano, l'appareil de Golgi se présente sous la forme de cordons anastomosés en réseau. D'autre part, on observe souvent une polarisation évidente de cet appareil de Golgi. Ces résultats sont particulièrement constants chez le Chat. Chez le Cobaye et le Rat, l'appareil de Golgi est moins nettement et moins frequemment polarisé, ceci étant en relation possible avec le fait que ces animaux presentent des cellules poins riches

en granules neurosécrétoires.

Le chondriome parait souvent difficile à mettre en évidence chez le Chat et le Cobaye. Il semble se présenter sous la forme de très fines mitochondries disséminées dans tout le cytoplasme. Chez le Rat, le chondriome est particulièrement développé et se présente également sous forme de mitochondries nombreuses, parmi lesquelles on rencontre quelques courts chondriocontes.

En conclusion, l'équipement cytologique des cellules neurosécrétoires de l'hypothalamus parait adapté à la synthèse de produits de sécrétion.

Référence bibliographique:

Romieu, M., A.Stahl et G. Cotte - 1953 - Cytologie des cellules nerveuses de l'hypothalamus. Acta Anatomica, vol. 18, p. 74.

No.

S. Zuckerman

(Department of Anatomy, University of Birmingham, England).

Hypothalamic anterior pituitary relations.

Recent evidence suggests that the hormones of the posterior lobe of the pituitary are secreted in the nuclei supraopticus and paraventricularis of the hypothalamus, from which they move by axonal transport into the infundibular process. In this sense, and in this manner, the hypothalamus directly controls the function of the posterior lobe. In some way the hypothalamus also controls that of the anterior lobe. An example of this control is the release of gonadotrophic hormone from the pars distalis following light stimulation of the retina in the ferret, or stimulation of the accessory reproductive organs in the rabbit; for in both cases the presumption is that the afferent impulses are relayed to the hypothalamus before the pituitary is affected. The evidence that axons of hypothalamic nuclei terminate amongst the glandular cells of the adenohypophysis is, however, equivocal; and the view is also general that those nerve fibres, whether sympathetic or parasympathetic, which reach the gland along its blood-vessels are vasomotor and not secretomotor in function. For this reason it is not believed that the way in which the hypothalamus controls the pars distalis is by nervous stimulation, or, as in the case of the posterior lobe, by the passage into the gland of some neurosecretion down the axons of hypothalamic neurons.

The alternative view has been advanced that neurosecretory material is transported from the hypothalamic nuclei to the pars tuberalis, where it is picked up by the primary capillary loops of the pituitary-portal

vessels. The neurosecretion is then supposed to pass down into the sinusoids of the *pars distalis*, and so to come into contact with the surrounding secretory cells. This thesis is not based upon direct evidence, but has been taken as a conclusion implied by a variety of collateral observations. These can be classified as follows:

(a) Stimulation of the hypothalamus

Lesions of the hypothalamus may be associated with hypoactivity and with other derangements of reproductive and sexual function. Conversely, stimulation of different hypothalamic areas may induce such positive effects as ovulation, due to the release of gonadotrophin, or lymphopenia, due to the presumed release of ACTH. Such observations do not show how changes in the activity of the hypothalamus influence the secretion of the anterior pituitary, but merely confirm that the anterior lobe can respond to changes in hypothalamic stimulation. In this sense they do not get us further forward in understanding exactly how the hypothalamus exerts its influence on the *pars distalis*.

(b) The behaviour of grafts of the pars distalis

Pituitary tissue can be homografted into the anterior chamber of the eye and into other regions of the body of hypophysectomized animals. Homografts rarely take properly, and as a rule the adrenal cortex in the host animals becomes greatly involuted, although not as much as in hypophysectomized controls. More promising results have been reported with autografts. Recently, Harris & Jacobsohn (1952), using closely-related rats as donor-host pairs, have shown that good union of the pituitary can be obtained in several sites, but that only grafts placed immediately under the median eminence, where they acquire a vascular connection with the primary loops of the pituitary-portal vessels, function properly. This observation plainly indicates that the chances of pituitary grafts surviving

are much enhanced if the donor and host are closely related genetically, and if the pituitary re-establishes contact with the anterior part of the floor of the hypothalamus, but it does not prove that the function of the pituitary-portal vessels is to transmit some chemical stimulant from the hypothalamus to the *pars distalis*, and that the function of the anterior lobe is dependent on the integrity of the vessels.

An observation which opposes this thesis is the fact that ACTH can be liberated from pituitary tissue that has been grafted into the anterior chamber of the eye.

(c) The effects of sections of the pituitary stalk

Many workers have reported on the effects of stalk section on pituitary function. The results obtained have been very variable, and it has been suggested that the pituitary-portal vessels have regenerated in those cases where pituitary function is either restored or remains normal. Harris (1950) has, for example, shown that the return of reproductive function in rats in which the pituitary stalks are divided is very closely correlated with the amount of regeneration of the hypophysial portal vessels.

In corresponding experiments reported by Greep & Barrnett (1951) and Barrnett and Greep (1951), regeneration of the pituitary-portal vessels was, however, not observed, although evidences of hyposecretion of gonadotrophic hormone were noted. According to these two workers, the pituitary hypofunction which occurs after stalk section is due to infarction of the *pars distalis*, resulting from interruption of the superior hypophysial arteries and portal veins.

Recent experiments indicate that reproductive function can be normal in ferrets in which the pituitary stalk has been divided (Thomson and Zuckerman 1953). In England normal ferrets breed between March and August, and are

in anoestrus during the winter months. They can be brought into reproductive condition, and made to breed, during the winter months if they are exposed to artificial light during the hours of darkness. This response is dependent upon the presence of the retina, the anterior pituitary and the gonads. It has recently been found that it still occurs after section of the pituitary stalk, and after the insertion of a small plate of waxed paper between the distal and proximal segments of the stalk. The response to light occurred when the intervening plate had effectively interrupted all anatomical connections between the pars distalis and the floor of the hypothalamus, and had prevented the regeneration of any vessels.

These experiments suggest that although the evidence is clear that changes in hypothalamic stimulation can affect the secretion of the anterior pituitary, the pituitary-portal vessels are not an essential part of the mechanism that is involved.

Thomson, A.P.D. & Zuckerman, S. - 1953 - Functional relations of the adenohypophysis and hypothalamus. Nature, Lond., Vol. 171, p. 970.

The paper will appear in full in Biological Reviews (Cambridge).

No.

Ellen Thomsen

(Royal Veterinary and Agricultural College, Copenhagen, Denmark)

Experimental evidence for the transport of secretory material in the axons of the neurosecretory cells of *Calliphora erythrocephala* Meig.

In an earlier investigation (E. Thomsen, 1952) it was found that in the adult blowfly, *Calliphora erythrocephala*, a hormone produced by the medial neurosecretory cells of the brain controls the function of the *corpus cardiacum*, and probably that of the *corpus allatum*. This was tested on the development of the eggs.

The present study was undertaken in order to investigate if this hormone might reach the *corpus cardiacum-allatum* complex by travelling inside the axons of the neurosecretory cells.

By now secretory material has been demonstrated morphologically in the axons of neurosecretory cells (n. c.) in a number of different animals including some insects.

In *Calliphora* the nerves from the n. c. of the brain immediately after leaving the brain fuse with the *nervus recurrens* to form a single nerve, which we might call the cardiac-recurrent nerve. This nerve runs through the narrow neck to the *corpus cardiacum* + *ganglion hypocerebrale* situated in the foremost part of the thorax.

If there is a flow of secretory material in the axons of the n. c. of *Calliphora* it should be possible to block this flow. To this purpose 100 flies had their cardiac-recurrent nerve ligated. Three to seven days after the operation the surviving 48 flies were dissected.

In 12 cases only the nerve was unbroken. In 27 flies the inspection of the living fly with the binocular dissecting microscope showed that the proximal end of the nerve was swollen and had a pronounced bluish tinge. In 13 other flies the proximal end of the nerve was swollen and contained small bluish globules or lumps. In no case was the distal end of the nerve found to have a bluish tinge or to contain bluish lumps.

In a number of cases sections of the proximal end of the nerve, stained with chrome-hematoxylin phloxine, showed bright red, moniliform axons which terminated in conspicuous red bulbs. Small dark blue granules were also found.

It is interesting that in the cases in which bluish lumps were seen in the living nerve, the sections of these nerves showed stained aggregations of material. Furthermore it proved possible to demonstrate the piling up of the secretory material in the living ligated nerve by means of darkfield illumination.

When using the darkfield illumination the bluish tinge and the bluish lumps of the proximal end of the nerve stood out very clearly, so that it was possible to have it photographed.

The results of the ligating experiments, as seen in the stained and the living nerves, leave little doubt that normally a flow of secretory material takes place from the n. c. of the brain through the axons into the corpus cardiacum and that this flow is dammed up by the ligature.

By means of the darkfield illumination it was also possible to distinguish the secretory material in the axons of the normal, unligated cardiac-recurrent nerve. It is interesting that the living axons formed moniliform strings just as the axons of the n. c. in stained sections.

Secretory material was likewise found in some axons of the living nervi oesophagei and nervi allati.

As a control, a piece of the living abdominal nerve was studied with darkfield illumination. In no case did I find axons of an appearance similar to that of the abovementioned nerves.

As another control a number of flies had their abdominal nerve ligated. In these flies the proximal end of the nerve showed a pronounced swelling, but neither did it have a bluish tinge nor did it contain any bluish lumps.

The results of the experiments indicate that secretory material, which is built up in the n. c., moves through the axons of these cells into the corpus cardiacum and the corpus allatum. Very likely this is the way in which the n. c. normally control the two organs mentioned.

The result of the ligating experiments in Calliphora is very well in line with the findings of other authors (Hild, 1951; Stutinsky, 1951; Passano, 1951; E. Scharrer and Wittenstein, 1952; and B. Scharrer, 1952) who have studied the problem by cutting the axons from neurosecretory cells in different animals.

So by now there is strong evidence for the idea already advanced by B. and E. Scharrer in 1944, that secretory material produced in the pericaryon of n. c. migrates down the axons of these cells.

A more detailed paper will be published in the Journal of Experimental Biology in 1954.

See also:

Thomsen, E. - 1952 - Functional significance of the neurosecretory brain cells and the corpus cardiacum in the female blow-fly, Calliphora erythrocephala Meig. J. exp. Biol., vol. 29, p. 137.

No.

Mathias Thomsen

(Zoological Laboratory, Royal Veterinary & Agricultural College, Copenhagen, Denmark)

Observations on the cytology of neurosecretion in various insects (Diptera and Hymenoptera).

The neurosecretory cells of the brain have been studied in Calliphora and some species of Hymenoptera, mainly on sections stained with Gomori's chrome-hematoxylin phloxine method.

1. In Calliphora erythrocephala (Diptera, Cyclorrhapha) the neurosecretory cells (n.c.) in newly emerged females are difficult to distinguish, but in older females they are very distinct, containing granules which often stain dark blue with the chrome-hematoxylin of the Gomori stain, but in other cases are stained red by the phloxine.

There are two medial groups of n.c., each comprising about 8-10 cells, and two lateral groups each consisting of about 3 cells. The axons from the n. c. generally stain faintly red; in one case only a short section was distinctly blue with somewhat swollen axons.

In living n. c., photographed with darkfield illumination, the granules appear very strikingly as refractile white bodies.

2. The brains of several species of Hymenoptera have been examined with regard to the appearance of the n. c. In these insects the phenomenon of neurosecretion is extremely well developed. Slides of three species were presented: Vespa vulgaris (Fam. Vespidae), Synagris negus (Fam. Vespidae), and Sphecius speciosus (Fam. Stizidae). Others will be considered in the detailed paper to be published.

The three species mentioned agree in the general morphology of the neurosecretory system. There are two medial groups of n. c. comprising a rather great number of cells, the two groups lying very close to one another in the pars intercerebralis of the protocerebrum. Some of the n. c. contain distinct granules which stain dark blue with the hematoxylin of the Gomori stain, while in others the granules are less distinct or the cells stain a more uniform light red, presumably in accordance with the stage in the secretory cycle.

The axons of the n. c. stain blue. They are especially beautiful in Sphecius, where they can be followed the whole way from the perycarya of the n. c. to the corpora cardiaca. In some cases fine bead-like fibres are seen, and in other cases conspicuous swellings occur which may be accompanied by real lumps of dark blue material.

The nervi corporis cardiaci - the extracerebral parts of the fibre bundles from the n. c. - are short, as the two c. cardiaca lie close to the brain. These nerves are conspicuous by their content of blue axons which continue into the c. cardiaca as a well-defined central bundle in each. Arrived here, the axons diverge and branch between the cells of the c. cardiacum. The stained sections give the impression of a copious flow of secretory material from the brain into the c. cardiacum. The observations confirm the statements of Stutinsky (1952) and Hanström (1953) that the blue material belongs to the nerve fibres and does not enter the cardiacum cells. In Hymenoptera - like in several other insects - there are two types of cells: large cells of a simple roundish shape with large nuclei and conspicuous nucleoli, and small cells, sometimes spoken of as glia cells, which form a vacuolated tissue, somewhat resembling a mesenchyme. Both types stain red with the phloxine. Cellular processes could not be seen.

It is possible that the large cells of the *c. cardiacum* may have a secretory function of their own.

The *nervus corporis allati*, formed by some of the axons of the *n. corporis cardiaci* which continue into the *c. allatum*, contains a small amount of blue secretory material, but this seems to disappear in the *c. allatum* itself (*Synagris*).

The detailed paper will appear in: Det Kongelige Danske Videnskabernes Selskab, Biologiske Meddelelser, 1954.

~~Hanstrom, B. - 1953 - Neurosecretory pathways in the head of crustaceans, insects and vertebrates. Nature, London, 171, 72-75.~~

~~Stutinsky, F. - 1952 - Etude du complex retro-cerebral de quelques insectes avec l'hematoxyline chromique. Bull. Soc. Zool. France, 77, 61-67.~~

No.

J. J. Bounhiol, M. Gabe & L. Arvy

(Faculté des Sciences, Université de Bordeaux et Laboratoire d'Histologie comparée, Université de Paris, France)

Données histophysiologiques sur la neuro-sécrétion chez *Bombyx mori* L. et sur ses rapports avec les glandes endocrines.

L'étude histophysiologique de la neuro-sécrétion céphalique chez *Bombyx mori* L. (race Awoijku) permet, en complétant nos travaux antérieurs de préciser les points suivants:

1 - Il existe des cellules neuro-sécrétrices: a) dans le ganglion frontal, b) dans la partie médiale du proto-cerebron (*pars intercerebralis*), c) dans les parties latérales du proto-cerebron (à coté des *globuli* des *corpora pedunculata*) et d) dans le ganglion sous-oesophagien (partie ventrale et postérieure). La troisième de ces localisations n'existe pas chez la chenille qui, munie d'antennes et d'yeux rudimentaires n'a pas, comme les larves des Hémimétaboles, des lobes optiques prolongeant latéralement les ganglions cérébroïdes.

2 - Les éléments neuro-sécréteurs sont, cytologiquement, semblables, à part quelques détails indiqués, à ceux observés chez d'autres Insectes.

3 - Les cellules de la *pars intercerebralis*, constantes et les plus nombreuses dès la naissance subissent, au cours du développement post-embryonnaire, des modifications cycliques; à leur décharge correspond le cheminement du produit de sécrétion le long des axones. Fait nouveau: ce produit parvient non seulement aux

corpora cardiaca mais aussi aux corpora allata; il reste intercellulaire. La répartition des flaques colorées dans ces deux organes endocrines est la même que la répartition des terminaisons nerveuses établies antérieurement par d'autres méthodes: en particulier pour le corps allate on en observe entre les cellules dans la masse de l'organe et aussi sous la capsule.

Au dernier âge, après une longue phase de mise en charge (pendant la période d'alimentation obligatoire) s'observe une décharge qui se poursuit pendant la "montée" et le filage.

Une nouvelle mise en charge beaucoup plus rapide se produit pendant les 48 premières heures de la vie de la chrysalide; intense vers le quatrième jour, la décharge se prolonge jusqu'à la mue imaginale.

Chez l'imago, les cellules neuro-sécrétrices fonctionnent de façon différente suivant les sexes. Chez le ♂ elles restent au repos tandis que chez la ♀ existe une nouvelle mise en charge suivie d'une décharge se produisant peu avant la ponte (qu'il y ait, ou non, accouplement).

4 - Les cellules protocérébrales latérales n'apparaissent que chez la chrysalide jeune; elles persistent jusqu'à la mort de l'animal sans montrer, chez les sujets étudiés, de cycle sécrétoire net.

5 - L'arrivée du produit des cellules neuro-sécrétrices dans les corpora cardiaca et dans les corpora allata est suivie d'une augmentation du volume de ces organes par croissance des cellules et pas seulement par distension des espaces intercellulaires. De plus, dans le corpus cardiacum, l'arrivée de la substance d'origine

cérébrale colorée par l'hématoxyline chromique, est suivie de l'apparition, dans l'intérieur des cellules, d'une substance colorée par la phloxine.

6 - Les cellules neuro-sécrétrices du ganglion sous-oesopgagien semblent fonctionner de façon continue.

Le rapport in-extenso sera publié dans: Bulletin Biologique France-Belgique.

Travaux des auteurs à consulter:

Arvy, L., Bounhiol, J.J. et Gabe, M. - 1953 - Déroulement de la neuro-sécrétion protocérébrale chez Bombyx mori L. au cours du développement post-embryonnaire. C.R.Acad. Sc., Tome 236,p.627.

Bounhiol, J. J. - Travaux expérimentaux sur cerveau, ganglion frontal et c.a. . . . depuis 1936. Voir Bull. Biol. France-Belgique, Suppl. XXIV, 1938; Colloque international C.N.R.S., Paris, 1947; IX[e] Congrès international d'Entomologie, Amsterdam, 1951.

No.

Luigia Grandori & Enrica Care'

(Istituto di Entomologia agraria e Bachicoltura, Università degli Studi, Milano, Italia)

Studio anatomo-istologico sul sistema neurosecretore in *Musca domestica* e *Calliphora erythrocephala*.

Contrariamente a quanto hanno affermato precedenti Autori, e cioè che i *corpora cardiaca* nell'adulto di *Musca* e *Calliphora* sono ridotti a poche cellule fuse col ganglio ipocerebrale, abbiamo potuto dimostrare l'esistenza in queste due specie di *corpora cardiaca* ben sviluppati e distinti dal ganglio ipocerebrale. Di ciò abbiamo reso conto in una recente pubblicazione.* Per l'argomento qui trattato ci interessa la parte anteriore dei corpi cardiaci, formati da due lobi riuniti da un largo ponte; lobi e ponte abbracciano ventralmente e lateralmente il fascio nervoso formato dal nervo ricorrente fuso coi nervi paracardiaci. I lobi sono costituiti da cellule cromofobe, e in esse penetra un fascio di fibre neuro-gliali, accompagnate da numerose cellule satelliti e costituenti un vero plesso.

In *Calliphora erythrocephala*, dopo 3 giorni dallo sfarfallamento, i corpi cardiaci si fanno turgidi, e le cellule cromofobe dei grandi lobi contengono una sostanza cromofoba e leggermente acidofila che si presenta a masserelle quasi regolari. In *Musca domestica* i corpi cardiaci sono ben

sviluppati negli ultimi giorni della ninfosi e sostanzialmente formati similmente a quelli di Calliphora. In piena attività riproduttiva degli adulti le cellule cromofobe dei lobi dei corpi cardiaci contengono masse cospicue di una sostanza leggermente acidofila, mentre nel plesso interno ai lobi, formato dalle fibre nervose floxinofile e dalle cellule satelliti, si distinguono nettamente formazioni colorabili con l'ematossilina cromica secondo Gomori. Tali formazioni però non hanno l'aspetto di masserelle compatte come quello degli increti delle cellule nervose della pars intercerebralis, bensì di gocce, filamenti, rivoletti che pare interessino le cellule satelliti ** e i loro prolungamenti citoplasmatici.

Nella pupa di Calliphora erythrocephala al 3° giorno dopo la formazione del pupario, le cellule nervose della pars intercerebralis sono fortemente vacuolizzate e nessuna di esse si colora con l'ematossilina cromica. Una decisa colorazione di tali cellule con l'ematossilina cromica abbiamo ottenuto nella femmina adulta 5 giorni dopo lo sfarfallamento. Esse sono in numero limitato in vicinanza a cellule nervose più grandi, a citoplasma floxinofilo che non si colorano con l'ematossilina cromica secondo Gomori. Nessuna indicazione abbiamo trovato finora in Calliphora sulla possibilità della conduzione della sostanza colorabile con l'ematossilina cromica dalle cellule nervose ai corpi cardiaci.

Osservazioni simili abbiamo compiuto sulla Musca domestica

in piena attività riproduttiva. Anche nella Mosca allo stadio di pupa non abbiamo ottenuto una colorazione con l'ematossilina cromica delle cellule nervose della pars intercerebralis. Soltanto al 5° e 6° giorno dopo lo sfarfallamento si notano alcune cellule nervose della pars intercerebralis che si colorano nettamente con l'ematossilina cromica. I granuli dell' increto blu-nerastri sono in questo periodo ancora piccoli e abbastanza distanziati, ma nelle piena attività riproduttiva della Mosca, e cioè dopo 15-17 giorni dello sfarfallamento, le cellule neurosecretrici sono piene di masserelle compatte colorate dalla Gomori. Tali cellule non sono numerose e si trovano o incuneate fra le altre cellule nervose, o addossate ad esse in posizione più superficiale, ma sempre si distinguono dalle grandi cellule motrici che nella sezione parasagittale appajono disposte ad arco attorno al neuropilo, e che hanno di solito citoplasma ricco in ribonucleina. Osservati infine, tanto in Calliphora che in Musca, gli assoni provenienti dalle cellule colorabili con l'ematossilina cromica della pars intercerebralis, e che costituirebbero le radici dei nervi paracardiaci, non abbiamo lungo di essi trovato alcuna prova della presenza di formazioni colorabili con l'ematossilina cromica. Soltanto all'entrata delle fibre neurogliali nei corpi cardiaci sono nettamente visibili formazioni colorabili con l'ematossilina cromica.

Ci troviamo, secondo noi, davanti a 3 sorta di sostanze elaborate dal sistema neurosecretore (formato dalle cellule neurosecretrici della pars intercerebralis, dei nervi paracardiaci

e dai corpi cardiaci): 2 increti che si colorano con l'ematossilina cromica, l'uno nelle cellule nervose della pars intercerebralis, l'altro nelle cellule dei lobi dei corpi cardiaci; il 3° elaborato appare nel plesso fibro-gliale interno ai lobi dei corpi cardiaci.

---

* Grandori, L. e Care' E. - 1953 - I corpi faringei (corpora cardiaca) in Calliphora erythrocephala Mg. adulta. Boll. Zool. Agr. e Bachic., vol. 19, 1.

** Abbiamo preso in considerazione, oltre alle cellule nervose, le cellule satelliti o accompagnatrici (cellule gliali ?) che sono numerose lungo le fibre nervose che penetrano nei corpi cardiaci.

No.

Francis G. W. Knowles *

(Marlborough College, Wilts, Wiltshire, England)

Neurosecretion in the tritocerebral complex of crustaceans.

The first clear indication that nervous tissues of crustaceans might contain pigment-activating hormones was given by Brown (1933) who found that the injection of an extract of the central nervous system was followed by the concentration of dispersed red pigments in the chromatophores of eyestalkless animals. Later experiments showed that the nerve cord in the thorax contained appreciable quantities of a white-pigment-concentrating hormone, and that the presence of this hormone could not be attributed to sinus-gland activity (Knowles, 1939). Ablation experiments (Knowles, 1952) finally eliminated the possibility that the sinus-glands produced the white-pigment-activator in crustaceans.

In 1940 Brown and Ederstrom directed our attention to the circum-oesophageal connectives as a very potent source of a hormone which dispersed black pigment in the telson and in the uropods of Crago, and in 1941 Brown and Wulff extracted white- and red-pigment-concentrating hormones from the circum-oesophageal connectives. In later experiments it was found that the greatest "tail-darkening activity" in Crago lay not in the connectives, but in the post-oesophageal commissure, especially near two slight swellings of the commissure in which a bluish-green particulate substance could be seen and some cell-bodies could be detected (Brown, 1946).

---

* The recent research work described in this report has been aided by grants from the Royal Society and from the Nuffield Foundation. I am greatly indebted to these two bodies for their financial support which has made much of this work possible.

The post-oesophageal commissure is a very constant feature in most of the groups of the higher crustacea. Hanström and others have shown that it should be considered as part of the tritocerebrum and that it, the connectives and their ganglia represent part of the nervous system of the first post-oesophageal somite which has secondarily come into relation with the brain. A detailed account of this region has recently been published (Knowles, 1953), in which the course of two fine nerves which leave the hinder margin of the commissure is described, and in which the interpretation of these nerves given by Police (1908), and followed by later authors, is shown to be inaccurate.

In the crustaceans studied each post-commissure nerve innervates a muscle which is attached at its base to the cephalic apodeme of the endophragmal skeleton and dorsally by a long tendon to the dorsal hypodermis. It is suggested that these muscles may act as "moulting muscles", or that by altering the pressure of body fluids they may control the flow of blood in the right and left branchial sinuses.

Evidences of neurosecretion are found in the two post-commissure nerves of the genera Squilla, Penaeus and Leander. Considerable quantities of fuchsinophil droplets are found along the course of these nerves and in two lamellae each of which is formed by an extension of the epineurium at some point along the course of the nerve. In the Penaeids (P. braziliensis and P. caramote) examined each epineurium extension takes the form of a disc 5-7 μ in thickness and 150 μ in diameter, which arises at the point where each post-commissure nerve reaches the muscle that it innervates. In the stomatopod Squilla the median portions of the post-commissure nerves are expanded to form two irregularily shaped lamellae which lie on the surface of two oesophageal muscles. In the members of the Palaemonidae examined

(Leander serratus, L. affinis) the proximal portions of the post-commissure nerves are expanded to form two irregularily shaped lamellae which are fused by a horizontal bar at some distance from the commissure.

The epineurium extensions of the post-commissure nerves are thus diverse in form in different crustacean groups but they have these features in common: (a) Each contains fuchsinophil droplets and also an intricate meshwork of fine fibre terminations, forming a neuropile-like mass. (b) They bear a close spatial relationship to the blood system. In Penaeus the post-commissure lamellae are actually fused to the wall of a blood sinus in which blood travels from the gills. The features enumerated above are also shared to some degree by the sinus-glands and by other neurosecretory organs in crustaceans and the term "neurohaemal organ" is proposed (Carlisle and Knowles; in press) to describe any system of fibre terminations in crustaceans, which bears a close spatial relationship to the blood system, and is by inference possibly neurosecretory.

Each post-commissure nerve contains two large motor fibres which innervate the dorso-ventral muscle which the nerve supplies. These fibres originate in two large cell-bodies 75 μ in diameter in which the generally accepted evidence of neurosecretion has been observed in Penaeus; droplets in the cytoplasm stain with the phloxine component of the Gomori chrome-haematoxylin-phloxine stain. If these droplets are indeed neurosecretory then the neurones in which they are found serve a dual purpose as motor neurones innervating a muscle and as centres of secretion.

The commissure contains cell-bodies within its hinder margin close to the points of emergence of the post-commissure nerves (see Knowles, 1953). As yet no fibre components of these cells have been detected by the usual

staining methods, though in other respects these cells resemble the neurone cell-bodies found elsewhere. Spherical or oval vacuoles in the nuclei and in the cytoplasm of these cells may indicate the presence of secretory material which has been washed out during the process of fixation. It is hoped that this hypothesis may later be tested by observations on living cells.

The origin of the fine branched fibres which terminate in the neurohaemal organs is still uncertain. In Penaeus caramote these fibres have been followed along the hinder margin of the commissure and forwards as far as the connective ganglia, but their cell bodies have not yet been identified. The possibility that these fibres are not neurosecretory but that they "trigger" the release of secretory substances from the neurohaemal organs is being investigated.

The functional significance of the post-commissure neurohaemal organs has been studied by injection experiments, using the movements of pigments in the chromatophores as indication of the presence of pigment-activating hormones. By these means it has been shown that the pigment-activating hormones of the commissure region are most densely concentrated in the neurohaemal organs. A number of pigment movements have been observed after injection of extracts of the post-commissure neurohaemal organs and it has been shown that these movements differ from those which follow the injection of sinus-gland extracts.

Four pigment movements have been studied in Penaeus braziliensis and in Leander serratus. (1) The concentration of red pigment in large dark chromatophores (in Leander these form the bands of colour on the body); (2) the concentration of red pigments in small dark chromatophores; (3) the concentration of white pigments in light-reflecting chromatophores; (4) the

dispersal of red pigments in the chromatophores of the telson and the uropods. For convenience the substances causing these effects will be referred to her as RL, RS, W and DT respectively.

In *Leander* the sinus-gland has been shown to contain RS. The post-commissure neurohaemal organs contain RL, W and DT. There is evidence that in *Penaeus* RL and W are separate substances. Brown and Saigh (1946) have put forward evidence that DT is not the same substance as those which concentrate the body chromatophore pigments. It seems likely therefore that there are at least four different pigment-activating hormones in crustaceans and that three of these are released through the post-commissure neurohaemal organs.

It has long been known that sinus-gland extracts were without effect on the chromatophores of the stomatopods. Recently it has been found that the post-commissure neurohaemal organs appear to be the main centres of pigment-activating hormones in *Squilla mantis*. Apparently these organs contain only two of the pigment activating principles listed above, namely DL and W. This has been determined by the reciprocal injection experiments represented by Table 1.

To summarise, it seems clear that the circum-oesophageal and post-oesophageal regions of the tritocerebrum in crustaceans contain cells which manufacture pigment-activating hormones which are released into the blood stream through flattened portions of the two post-commissure nerves. The substances which are produced play an important part in the colour-change of many crustaceans and in one group at least the tritocerebrum seems to be the only source of colour-change hormones. But many problems remain. We do not yet know the chemical nature of the hormones, nor how they are transported from the cells which manufacture them to the points where they are released. The fine

branched fibres which permeate every part of the post-commissure neurohaemal organs may bring the hormones to these organs or on the other hand they may control hormone release. There are still some chromatophore pigments, the yellow for instance, for whose movements we cannot yet account fully in terms of neurosecretory hormones. Our concept of the hormonal control of colour changes in crustaceans is very different from what it was a few years ago, largely because of advances in the field of neurosecretion, but each advance brings both opportunities and problems, and this is very evident in the study of crustacean colour-change.

Knowles, F.G.W. - 1939.- Pubbl. Staz. Zool. Napoli, 17: 174.

Knowles, F.G.W. —1952.- Physiol. Comp. et Oecol., 2: 289.

Knowles, F.W.G. - 1953.- Proc. Roy. Soc., 141: 248.

Carlisle, D. B. and Knowles, F.G.W. - Nature. (in press).

Origin of extract	Species of injected animal				
Squilla	Squilla		Leander serratus		
	Red	White	Large Red	Small Red	White
Sinus gland	-	-	(+)	+	-
Eye-stalk	(+)	-	(+)	(+)	-
Brain	(+)	-	(+)	(+)	-
Connectives	(+)	-	(+)	-	-
Connective ganglia	(+)	-	-	-	-
Post-commissure neurohaemal organs	++ ++	++ ++	++ ++	-	++ ++
Thoracic ganglia	-	-	(+)	-	-

Table 1. The comparative distribution of pigment-activating hormones in the nervous system and neurohaemal organs of Squilla mantis. Extracts were tested for their effects on the red and white pigments of the chromatophores by injection experiments.

(+) denotes an incomplete concentration which lasted for an hour or less.

\+ denotes a complete concentration which lasted for an hour or less.

++ denotes a complete concentration which lasted for longer than an hour.
++

Dorothy E. Bliss, James B. Durand & John H. Welsh

(The Biological Laboratories, Harvard University, Cambridge 38, Massachusetts, U.S.A.)

Some Decapod Crustacean Neurosecretory Systems.

The hypothesis that secretory material synthesized within the bodies of neurosecretory cells can migrate along the axons of these cells to storage-release centers composed of their endings has proved useful in the interpretation of certain observations made upon the Crustacea by several investigators. Our own experiments on the land crab, *Gecarcinus lateralis* (Fréminville), revealed that the rise in respiratory rate, the fall in respiratory quotient, and the uptake of water, all of which follow eyestalk removal and indicate approaching molt, do not occur after removal of the sinus glands alone. These observations suggested that in the decapod Crustacea the sinus glands are acting as reservoirs of molt-inhibiting hormone produced by groups of neurosecretory cells located elsewhere in the eyestalk. This interpretation was confirmed by morphological studies on *Gecarcinus* and other brachyurans. Furthermore, the fact that neurosecretory cells are widely distributed throughout the central nervous system of *Gecarcinus* and that axons of many of these cells can be traced to the sinus glands suggested that these organs are serving as storage-release centers for secretory products not only from neurosecretory cells of the eyestalk but also from similar cells in the brain and probably even in the thoracic ganglionic mass. There is evidence that the sinus glands are formed by the massed swollen endings of these neurosecretory cell fibers.

Recent work has shown that such a neurosecretory system is not confined to the Brachyura but that it exists in much the same form in the crayfish, Cambarus virilis Hagen. The sinus glands of the crayfish, like those of the land crab, consist of the fiber endings of neurosecretory cells located in the eyestalks, brain, and undoubtedly the ventral ganglia. "Maps" showing the regions of the eyestalks and the brain in which neurosecretory cells are found have been drawn from reconstructions of tissues fixed in Helly's and stained with Gomori's chrome-hematoxylin-phloxin. In these species the regions containing neurosecretory cells are found in corresponding parts of eyestalks and brain. Four regions are distinguishable in the eyestalks of Gecarcinus and five in those of Cambarus, while the brain of both species contains five regions. Fiber tracts from neurosecretory cells in these regions run along parallel paths to the sinus glands. Differences of detail exist - for example, there is fusion of two regions in one species and not in the other - but a basic morphological similarity is clearly apparent. Analogous regions of neurosecretory cells in these "maps" have been identically labeled. It is hoped that the "maps" may guide future cytological and physiological research on neurosecretion in these species and in other decapod Crustacea.

A complete account of this work is appearing in the "Zeitschrift für Zellforschung."

Recent papers on the subject include:

Bliss, D.E. and Welsh, J.H. - 1952 - The neurosecretory system of brachyuran Crustacea. Biol. Bull., Vol. 103, p. 157.

Bliss, D.E. - 1953 - Endocrine control of metabolism in the land crab, Gecarcinus lateralis (Fréminville). I. Differences in the respiratory metabolism of sinusglandless and eyestalkless crabs. Biol. Bull.,

No.

Lucie Arvy & Manfred Gabe

(Laboratoire d'Anatomie et Histologie comparées, Sorbonne, France)

Données histophysiologiques sur la neuro-sécrétion chez les Insectes Paléoptères (Ephéméroptères et Odonates).

L'étude histophysiologique des systèmes neuro-sécréteurs céphaliques chez 30 espèces d'Ephéméroptères* et 4 espèces d'Odonates montre les faits suivants:

1. Les Paléoptères étudiés ont 3 systèmes neuro-sécréteurs céphaliques. Deux d'entre eux ont leur péricaryones situés dans la pars intercerebralis du protocerebrum et près des globuli des corpora pedunculata; leurs axones forment les nervi corporis cardiaci qui innervent le corpus cardiacum et le corpus allatum chez les Odonates et seulement le corpus cardiacum chez les Ephéméroptères. Le troisième système neurosécréteur est représenté par des cellules neurosécrétrices situées dans le ganglion sous-oesophagien et dont les axones innervent les corpora allata et la glande ventrale chez les Ephéméroptères et seulement la glande ventrale chez les Odonates.

2. Les caracteres cytologiques des cellules neuro-sécrétrices des Paléoptères sont les mêmes que chez les autres insectes; le produit de secretion s'accumule dans le cytoplasme progressivement et proportionellement à la diminution des corps de Nissl et il flue hors de la cellule, le long

---

* Fixées à notre intention par le Dr. MACAN (d'Ambleside, Angleterre), que nous tenons à remercier bien vivement ici.

des axones. Ce produit arrive dans le corpus cardiacum et peu de temps après un deuxième produit de sécrétion apparait dans les cellules du corpus cardiacum; le corpus cardiacum n'est pas seulement un centre de stockage (storage): le produit de sécrétion élaboré par les cellules de la pars intercerebralis s'accumule entre les cellules du corpus cardiacum, mais les propres cellules de ce dernier organe élaborent un produit qui diffère de la sécrétion protocérébrale par ses caractères de solubilité et par son comportement vis-à-vis des colorants histologiques. C'est ce dernier produit qui passe dans le sang, à travers la paroi du vaisseau dorsal.

3. Le maximum d'activité des cellules neurosécrétrices protocérébrales se situe au moment de la mue subimaginale chez les Éphéméroptères et de la mue imaginale chez les Odonates. Peu de temps après la réalisation de cette sécrétion maxima, les organes qui sont innervés par les cellules neuro-sécrétrices du protocerebrum atteignent leur taille et leur activité les plus grandes.

4. L'activité maxima des cellules neuro-sécrétrices du ganglion sous-oesopgagien survient au cours de la seconde moitié de la vie larvaire; la diminution de la neuro-sécrétion est suivie de l'atrophie des organes qui sont innervés par les axones issus de ces cellules, c'est-à-dire les corpora allata et la glande ventrale chez les Éphéméroptères et la glande ventrale chez les Odonates.

5. Il existe donc un parallélisme évident entre l'évolution chronologique de l'activité neurosécrétrice et l'état des organes qui sont innervés par ces cellules.

Le travail original, accompagné d'illustrations, paraîtra dans la Zeitschrift für Zellforschung, Bd. . ., 1953.

No.

M. Dupont-Raabe

(Laboratoire de Zoologie, Sorbonne, Paris, France)

Le role endocrine du cerveau dans la regulation des phenomenes d'adaptation chromatique et de la ponte chez les Phasmides.

(No abstract submitted)

Editor's note: According to the author certain evidence indicates that the brain of phasmids contains a chromatophorotropin which is not localized in an area containing neurosecretory cells. At the time of egg deposition female phasmids show a particularly intense neurosecretory activity in their pars intercerebralis. However, the production and deposition of eggs in these insects can take place in the absence of the brain, a result which is in contrast to that in Calliphora (E. Thomsen, 1952). For further information on these subjects see:

Dupont-Raabe, M. - 1952 a - Substances chromactives de crustaces et d'insectes. Activite reciproque, repartition, differences qualitatives. Arch. zool. exper. gen, vol. 89, p. 102.

Dupont-Raabe, M. - 1952 b - Contribution a l'etude du role endocrine du cerveau et notamment de la pars intercerebralis chez les phasmides. Arch. zool. exper. gen. vol. 89, p. 128.

No.

B. Possompes

(Laboratoire de Zoologie, Sorbonne, Paris, France)

Donnees experimentales sur l'activation de l'anneau de Weismann par le cerveau chez Calliphora erythrocephala.

(No abstract submitted)

Editor's note: The main result of this investigation concerns the endocrine control of metamorphosis in the fly, Calliphora erythrocephala. A factor originating in neurosecretory cells of the brain stimulates the equivalent of the prothoracic gland (large ring gland cells, peritracheal gland) which in turn gives off the growth and differentiation hormone. This mechanism is in line with those in other insect species, except for the fact that in Calliphora stimulation of the peritracheal gland requires the nervous connection with the brain to be intact. One must conclude that in this case the hormone furnished by the neurosecretory cells reaches its destination (the peritracheal gland) by means of axonal transport, while in Lepidoptera and Hemiptera this takes place via the circulation. For details see:

Possompes, B. - 1953 - Recherches experimentales sur le determinisme de la metamorphose de Calliphora erythrocephala Meig. Arch. zool. exper. gen., vol. 89, p. 203.

No.

O. Koller

(Zoologisches Institut, Universitat des Saarlandes, Saarbrucken)

Neurosekretorische Steuerung der Darmbewegungen von Insekten.

(No abstract submitted)

Editor's note: The author reported on experiments which demonstrate the presence of humoral factors in the central nervous system of insects and crustaceans which affect intestinal motility. Extracts from insect brains or crustacean ganglia decrease the frequency of the muscular contractions in the isolated intestine of insects, such as Tenebrio. Eyestalks from dark-adapted crustaceans act in the same way, while those from light-adapted donors have the opposite effect (see also Hara, 1952). Whether or not these results can be attributed to neurosecretory activity is as yet uncertain, since comparable myotropic factors can be extracted also from non-nervous tissues.

No.

V. B. Wigglesworth

(Department of Zoology, University of Cambridge, England)

Neurosecretion and the corpus cardiacum of insects

Neurosecretory cells and moulting in Rhodnius (Hemiptera).

It was first shown by Kopeć (1917, 1922) that the brain is the source of the secretion necessary to induce pupation in Lepidoptera; and this was later confirmed by Caspari and Plagge (1935). In Rhodnius the same experimental result was obtained: removal of the head before a certain critical period in the instar prevents moulting; and moulting is induced in these decapitated insects by transfusion of blood from larvae that have passed the critical period (Wigglesworth, 1934).

At first I suggested that this hormone which induces moulting might be secreted by the corpus allatum, which shows signs of activity at this period; but I could obtain no good experimental evidence of this (Wigglesworth, 1936). Later, after Professor Hanström had kindly examined the brain in Rhodnius and described the presence of neurosecretory cells in the pars intercerebralis (Hanström, 1938) I found that this region of the brain, excised after the critical period and implanted into the abdomen of Rhodnius larvae decapitated soon after feeding, would cause them to moult, whereas no other part of the brain or suboesophageal ganglia and neither the corpus allatum nor the corpus cardiacum has this effect (Wigglesworth, 1940).

At about this same time it was shown by Fukuda (1940, 1941, 1944) that in both larva and pupa of the silkworm, the prothoracic gland is the immediate source of the growth or moulting hormone; and later Williams (1947, 1948)

proved that secretions from the neurosecretory cells in the brain are necessary to activate the prothoracic glands.

During the past few years evidence has been accumulating that this two-stage mechanism may be general among insects. In cyclorrhaphous Diptera it has long been recognised that the hormone causing pupation is produced in the ring gland of Weismann (Burtt, 1937, 1938; Hadorn, 1937) and almost certainly by the large lateral cells of this gland (Vogt, 1943) which are now commonly regarded as homologous with the "pericardial glands" (or prothoracic glands) of other insects (Thomsen, 1941). It has recently been proved by Possompès (1953) that these lateral cells (called by him the "peritracheal gland") are induced to secrete the pupation hormone by a factor coming from the brain.

It has been found that the same mechanism operates in Rhodnius. The immediate source of the hormone which initiates growth and moulting is a thoracic gland consisting of large cells with lobulated nuclei deeply buried in the fat body of the meso- and metathorax. These cells are activated by the factor secreted in the dorsum of the brain. Thus implantation of the thoracic gland from a larva which has just passed the critical period will induce moulting in the isolated abdomen of a recently fed larva; whereas implantation of the brain is effective only if the thorax is intact (Wigglesworth, 1952).

Now the histological study of neurosecretion in the cockroach Leucophaea (Scharrer, 1952) has shown that the secretory product of the neurosecretory cells of the protocerebrum passes along their axons to the corpus cardiacum, where it accumulates between the cells. Any consideration of the function of the neurosecretory cells in the brain therefore necessarily involves a

discussion of the function of the corpus cardiacum and of the secretion from the brain which accumulates within it.

An active substance from the corpus cardiacum of the cockroach (Periplaneta)

I propose to describe here some results obtained recently by Mr. M. L. Cameron working in my laboratory. The starting point of this work was the observation of Koller (1948) that extracts from the head of a variety of insects will induce or will accelerate movements in the Malpighian tubules. When this observation was taken in conjunction with the discovery of M. Thomsen (1943) and of Brown and Meglitch (1940) that the corpus cardiacum of insects contains a principle active on the chromatophores of many Crustacea, and with the work of Dupont-Raabe (1949, 1952) on the corpus cardiacum and colour change in insects, it occurred to me that these various activities might be the product of a single substance and that the corpus cardiacum might be concerned in the secretion, not of growth hormones, but of a pharmacologically active principle or principles comparable with those produced by the pituitary or suprarenal glands of vertebrates.

Cameron has carried out a wholly independent study of this problem. The investigation is not yet complete, but the results contained up to the present time are as follows (Cameron, 1953). He quickly showed that extracts from the corpus cardiacum of the cockroach (Periplaneta) cause increased movement in the Malpighian tubules of Locusta: one pair of glands extracted in 1 ml. will increase the rate of contraction at about 18°C from 18 per minute to 24 per minute and the strength of the movement is increased.

Search has been made for other reactive organs. The hind-gut of Locusta or Periplaneta isolated in Ringer's solution beats for hours with slow steady contractions. The effect of the corpus cardiacum extract from

Periplaneta is to accelerate the rate of contraction and also to increase the amplitude. On withdrawal of the extract it reverts to the former rate and amplitude. On the fore-gut of Periplaneta the extract has little effect on the rate of contraction but causes some depression of amplitude.

The most useful test organ has proved to be the heart. The detailed work of Krijgsman (1952) and others has shown that the insect heart reacts to cholinergic and adrenergic drugs; but no active substance produced by the insect itself has been demonstrated hitherto.

The heart-beat in Periplaneta was studied by the method of Krijgsman and Krijgsman-Berger (1951), slightly modified. Extracts from the corpus cardiacum of Periplaneta containing 1 pair of glands in the 10-15 ml. will increase the rate of heart beat by 50 per cent: smaller amounts will cause smaller increases, and even when the dilution is so great as to cause no change in frequency the amplitude is still increased.

The volume of a pair of glands from Periplaneta is about 0.04 mm^3. In 10 ml. of solution this is a concentration of 1:2-300,000. The gland cannot contain more than 10 per cent of active material, so we have a final concentration of one in two or three millions. The amplitude is still noticeably affected at one tenth of this concentration. These compare well with the concentrations used by M. Thomsen and others working on chromatophores.

These same extracts were tested on the prawn Leander serratus and proved highly active in causing a violent concentration of the red and yellow chromatophores and a dispersal of the blue, converting a grey or dirty brown animal into a clear pale blue. (These effects resemble those described by Dupont-Raabe, 1952)

Nature of the active principle.

Cameron has made substantial progress in the isolation and characterisation of this active substance. Of the many drugs that have been tested on the heart of the cockroach by Krijgsman and others, the only ones which seemed to have the same effects at similar concentrations were acetylcholine and adrenaline. Acetylcholine has not the stability of the active material from the corpus cardiacum. It therefore seemed more likely that it was related to adrenaline.

The chromaffin histochemical test as elaborated by Lison (1936) was carried out on whole preparations of the corpus cardiacum. This test consists in placing one gland in a solution of potassium dichromate and another in a solution of potassium iodate. If both develop a brownish yellow colour in the same way one may assume that the reaction is due to a diphenol. The corpus cardiacum was positive for this reaction, the yellow colour being localised in the rounded glandular part.

A method has been developed by James (1948) by which less than one gamma of adrenaline can be detected on a paper chromatogram. This method was used to study the material more closely. Extracts relatively free from contaminants were obtained by using von Euler's extraction process for adrenaline.

It has recently been claimed by Gregerman and Wald (1952) using the same methods, that no adrenaline could be found in Tenebrio larvae, but that there were at least two unknown orthodiphenols. Cameron has repeated their analysis of Tenebrio, but with one modification - the brei was extracted rapidly, instead of being allowed to stand overnight in the refrigerator.

The extract from Tenebrio treated in this way reveals three spots, the upper two corresponding to those described by Gregerman and Wald and a third between RF 0.3 and 0.4 which is quite diffuse and gives a pink colour with

the reagent. This is the active material. If eluted from the paper it has the activity of the corpus cardiacum extract; the other spots do not. If the same procedure is carried out on the corpus cardiacum extract of the cockroach, only this one spot is produced on the chromatogram.

Adrenaline treated similarly has an R_F of about 0.5, is coloured pink instantly by the reagent in the cold and becomes yellow on heating or standing. The active principle of the corpus cardiacum becomes colourless on standing. The only other substances of this group of which the R_F is near to that of the new active principle, are noradrenaline and dihydroxyphenylalanine (DOPA); on standing these are said to become brown and blue respectively.

Although the tests applied so far are not entirely specific, it thus seems probable that the active substance is an orthodiphenol. It is certainly not adrenaline, and almost certainly not one of the known orthodiphenols that are pharmacologically active. It must have a side chain that is capable of closing to form a coloured oxidation product, but closes less readily than in adrenaline.

Whether this active substance is identical with that studied by Thomsen (1943) to which the chromatophores of Leander are so sensitive, is still uncertain. Indeed, the most highly purified preparations, which were intensely active on the heart, seemed to have lost their activity on the chromatophores of Leander. The same substance has been demonstrated in the corpus cardiacum of Rhodnius, though in much smaller amounts than in the cockroach.

The active principle and the neurosecretory cells of the brain.

It has been shown by Scharrer (1952) in the cockroach Leucophaea that conspicuously staining material (termed "colloid" by her) passes down the

axons from the neurosecretory cells in the *pars intercerebralis* of the brain to the *corpus cardiacum*, and often beyond this to the aorta and hypocerebral ganglion. If the nerve to the *corpus cardiacum* on one side is cut, the "colloid" disappears from the gland on that side within a few days; whereas there is still a plentiful supply in the median neurosecretory cells and nerve tract and it may even accumulate above the severed end of the nerve.

Cameron has carried out unilateral nerve sections in *Periplaneta*. At 5 days and at 17 days after the operation the *corpora cardiaca* were removed and extracts prepared from organs with the nerve cut and organs with the nerve supply intact. In no case did section of the nerve lead to any reduction in the activity of the extract on the heart. *Cameron* is therefore of the opinion that this active principle is produced in the gland itself and not in the neurosecretory cells of the brain. Extracts from the brain, suboesophageal ganglion or *corpus allatum* have no action at all on the heart. On the other hand, in the single experiment made so far, an extract from a *corpus cardiacum* after the nerve had been cut had no action on the chromatophores of *Leander*.

These results throw no clear light on the function of the "colloid" from the neurosecretory cells which flows into the *corpus cardiacum*. It has been shown recently by *Arvy*, *Bounhiol* and *Gabe* (1953) that in the silkworm this substance flows not only to the *corpus cardiacum* but also to the *corpus allatum*. There is evidence also, as we have seen, that the secretion of the neurosecretory cells activates the prothoracic gland. Is it possible to regard the secretory product of the neurosecretory cells in the brain as some kind of labile raw material which is modified by the various endocrine organs that it reaches to give rise to their specific hormones?

Summary

The function of the neurosecretory cells of the brain in activating the thoracic glands of Rhodnius and other insects and so leading to the secretion of the growth or moulting hormone is briefly reviewed.

A preliminary account is given of recent work by M. L. Cameron on the corpus cardiacum of the cockroach.

The corpus cardiacum secretes an active principle which causes acceleration of the heart beat and of contraction in the hind-gut, and increases the movements of the Malpighian tubules.

This active principle is secreted in the corpus cardiacum itself and is not derived from the neurosecretory cells of the brain. Present evidence suggests that it is distinct from the chromatophorotropic hormone of the brain and corpus cardiacum.

This new active substance has been partially purified. It is probably an orthodiphenol, distinct from adrenaline and other known diphenols which are pharmacologically active.

Wigglesworth, V. B. - 1934 - The physiology of ecdysis in Rhodnius prolixus. (Hemiptera). II. Factors controlling moulting and metamorphosis. Quart. J. Micr. Sci., Vol. 77, p. 191.

Wigglesworth, V. B. - 1936 - The functions of the corpus allatum in the growth and reproduction of Rhodnius prolixus (Hemiptera). Quart. J. Micr. Sci., Vol. 79, p. 91.

Wigglesworth, V. B. - 1940 - The determination of characters at metamorphosis in Rhodnius prolixus (Hemiptera). J. Exp. Biol., Vol. 17, p. 201.

Wigglesworth, V. B. - 1952 - The thoracic gland in Rhodnius prolixus (Hemiptera) and its role in moulting. J. Exp. Biol., Vol. 29, p. 561.

Cameron, M. L. - 1953 - The secretion of an orthodiphenol in the corpus cardiacum of the insect. Nature, (in the press).

No.

Masashi Enami

(College of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama, Japan)

The functional significance of beta-neurosecretory cells in Sesarma.

The secretory activity of the large beta-neurosecretory cells constituting the X-organ in Sesarma haematocheir was re-examined in order to clarify the neurosecretory relationship between this organ and the sinus gland. Supravital staining with either Janus green or Nile blue sulphate demonstrated that the cells in question are responsible for the production of three kinds of secretory materials, i.e., fine granules and larger droplets, both of which take the dyes remarkably well, and masses of metachromatically stained colloid. Fine granules make their appearance in the cytoplasm independently of the occurrence of the other two kinds of secretory material, both of the latter differentiating simultaneously from large masses of poorly stained colloid material. All these secretory materials were found within the thick axons of the respective cells which form the X-organ-sinus gland tract, and also in the sinus gland where they were located either within enlarged nerve-fiber terminals or in adjacent connective tissue. Sections of the sinus gland stained after Masson showed the presence of the three kinds of neurosecretory material equally well. As the result of either extirpation of the sinus gland or transection of the X-organ-sinus gland tract, accumulation of the secretory materials in question took place in the connective tissue surrounding the proximal end of the cut tract directly following the operations. A minute sinus-gland-like formation occurred temporarily at the location of the operation. As the post-operative period

increased, the site of this formation shifted gradually towards the proximal portion of the medulla terminalis. This observation may be correlated with the partial retrograde degeneration of the cut tract. Regeneration of the tract seemed to take place starting approximately 25 days after the operations. At first, regenerating axons bearing the neurosecretory materials spread fanwise in the distal direction, then united to form a consolidated tract whose terminal portion showed the normal structure of the sinus gland. Histological observation of the sinus-gland-like formation occurring shortly after the operations afforded some information about the fate of the neurosecretory materials transported by way of the axons of the beta-cells. On the basis of the present study, results of the author's previous histological work (1951) were revised.

The full account of this work will appear in Zeitschrift für Zellforschung. For further information see:

Enami, M. - 1951 a - The sources and activities of two chromatophorotropic hormones in crabs of the genus Sesarma. I. Experimental analyses. Biol. Bull. Vol. 100, p. 28.

Enami, M. - 1951 b - II. Histology of incretory elements. Biol. Bull., Vol. 101, p. 241.

No.

B.De Lerma

(Istituto di Zoologia dell'Università di Bari e Stazione
Zoologica di Napoli, Italia)

Osservazioni sulla neurosecrezione in Hydrous piceus L.
(Coleotteri).

Nella pars intercerebralis del protocerebro degli adulti (immagini) dei due sessi di Hydrous piceus si osservano due gruppi di cellule nervose, del tipo dei grossi neuroni plasmatici, posti al di sopra del ponte protocerebrale, tra i calici dei corpi peduncolati, l'uno all'altro ravvicinato in prossimità della linea mediana. Ciascun gruppo è costituito di circa 20 elementi, di 30-35 μ di diametro, con grosso nucleo centrale, vescicolare, provvisto di cospicuo nucleolo acidofilo, cui aderisce la scarsa cromatina, raccolta in piccole zolle appiattite. Nei medesimi nuclei si osservano inoltre da 4 a 6 piccoli granuli sparsi di un materiale marcatamente Feulgen-positivo. Il corpo cellulare (pirenoforo) si continua in un lungo prolungamento o peduncolo citoplasmatico, diretto verso l'interno del protocerebro, da cui si origina il vero assone della fibra. Gli assoni corrispondenti ai detti due gruppi intercerebrali costituiscono le radici dei nervi cardiaci interni, diretti ai corpora cardiaca, il cui decorso nell'interno del cerebron dell'idrofilo è quello stesso già per altri insetti descritto da Hanström(1940).

Sia in maschi che in femmine in attività sessuale, ma specialmente in queste ultime, i citoplasmi di detti neurociti

DL 2

risultano chi più chi meno ripieni di un materiale di aspetto granulare che assume, dopo ossidazione permanganica, intensa colorazione bleu scura con l' ematossilina cromica secondo Gomori. Dopo fissazione in Bouin i granuli appaiano piuttosto grossi, addensati attorno al nucleo, un pò diradati alla periferia cellulare; essi sono presenti, più minuti e diradati, anche nel prolungamento citoplasmatico. Dopo fissazione in Helly, tale materiale colorabile con ~~col metodo di Gomori~~ (cromofilo, cioè, ~~verso~~ l'em. cromica, dopo ossidazione permanganica) appare più finemente ed omogeneamente granulare. I grossi neurociti della parte intercerebrale presentano, dunque, anche per l'idrofilo (come per molti altri insetti sino ad oggi studiati) i caratteri morfologici e tintoriali degli elementi neurosecretori (Scharrer e Scharrer, 1944).

In un medesimo gruppo intercerebrale si riscontrano, di solito, elementi in varie fasi di attività di secrezione: alcuni con citoplasma ricolmo di granuli di neurosecreto, altri a granuli più scarsi e diradati, altri ancora che non mostrano segni morfologici evidenti di secrezione, presentando citoplasma omogeneamente tinto in roseo-viola. Lo studio dei rapporti topografici tra neurosecreto e sostanza cromofila (corpo di Nissl) è facilitato dal confronto fra tagli alterni, colorati rispettivamente secondo i metodi di Gomori all'em. cromica e di Giemsa, di materiale fissato in una miscela a parti uguali di sol. acquose sature di ac. picrico e di sublimato (un fissatore adeguato per la conservazione della sostanza di Nissl). Dopo colorazione secondo Giemsa si osserva una caratteristica struttura a rete della sostanza cromofila, colorata in bleu, nelle cui maglie

è contenuto un materiale in forma di gocciole appena colorate in roseo pallido un pò violaceo, lo stesso materiale, verosimilmente, che assume l'intensa tinta bleu con l'em. cromica.

In corrispondenza delle superfici latero-dorsali del protocerebro si riscontrano due ulteriori gruppi di grossi neurociti plasmatici, ciascuno costituito di 4-6 grossi elementi (sui 35-40 μ), posti da un lato e dall'altro della parte intercerebrale, al di sopra delle cellule globulari dei calici, immediatamente al di sotto del perineurio cerebrale. Nel citoplasma di tali ultimi elementi, del tutto simili per i caratteri del nucleo a quelli intercerebrali, non si osservano segni morfologici netti di un'attività di neurosecrezione, almeno per lo stadio dello sviluppo immaginale qui preso in esame. Ulteriori ricerche al riguardo si rendono necessarie.

Il secreto prodotto dai neurociti intercerebrali decorre lungo gli assoni, secondo attesta la presenza di minute granulazioni colorabili col metodo di Gomori lungo talune delle fibre del tratto intercerebralis-cardiacus. Il neurosecreto si addensa nel segmento extracerebrale dei nervi cardiaci, in prossimità dei corpi cardiaci, e sembra decorrere alla periferia degli assoni, all'interno di quello strato di spessore submicroscopico, di natura lipoprotidica, che secondo Richards (1943, 1944) delimita la fibra nervosa degli insetti. In tal senso, si può pensare che il neurosecreto abbia decorso intra-neuronico, costituendo talora - su quei tratti delle fibre ove esso si va addensando - una sorta di crosta periassonica (cfr. Scharrer, 1952; Stutinsky, 1952; Bargmann, 1953).

Nei corpi cardiaci (= corpi faringei, De Lerma, 1933 *)

il neurosecreto si presenta come raccolto in cospicue masse intercellulari di deposito, colorabili con l'ematossilina cromica in vari toni di bleu scuro, sino al grigio, dopo prolungata differenziazione. Esso si accumula in prevalenza verso le superfici laterali esterne dei corpi, per versarsi nelle lacune linfoematiche latero-faringee. E' evidente l'origine cerebrale del detto secreto, secondo sostiene Scharrer (1951) per Leucophaea maderae, per i seguenti ordini di fatti: a) la localizzazione del secreto nei corpi è costantemente intercellulare, b) il suo comportamento di fronte all' ematossilina cromica è del tutto riportabile a quello che caratterizza il materiale colorabile col metodo di Gomori nei neuroni intercerebrali, c) nei citoplasmi, decisamente floxinofili, delle cellule dei c. cardiaci non si osservano mai segni di inclusioni colorate secondo il metodo di Gomori. D'altra parte, tra gli isolotti di cellule in cui è compartito il parenchima dei corpi, ed anche tra cellula e cellula, si riscontrano, frammiste ad altri del tutto incolori, segmenti di fibre fortemente impregnati di materiale colorato con ematossilina cromica che pare espandersi in gocciole e masserelle di deposito. Si tratta di un punto molto delicato della fine citologia dei fenomeni di neurosecrezione, su cui occorre condurre nuove indagini, con l'ausilio di metodi specifici per lo studio delle terminazioni fibrillari.

Nei corpi cardiaci si riscontra, oltre quella a carattere di neuro secreto ~~"Gomori-positivo"~~ dianzi descritta, un'altra secrezione, floxinofila, che si raccoglie in prevalenza verso le regioni laterali interne degli organi, per versarsi direttamente nell'aorta cefalica. Tale secrezione floxinofila, ben distinta dal neurosecreto di accumulo, parrebbe prodotta da cellule stesse dei corpi cardiaci, nei cui citoplasmi si osservano talora ampie inclusioni di un

materiale omogeneamente floxinofilo. Sono tuttora in corso mie ricerche al riguardo. Giova ricordare che fatti analoghi sono stati rilevati per i c. cardiaci di Efemerotteri, da Arvy e Gabe (1952) e di Carausius, da Stutinsky (1952). La presenza nei c. cardiaci di due distinti tipi di secreto, uno dei quali, floxinofilo, di molto probabile origine locale (l'altro, il neurosecreto, proven~~endo~~iente dal cervello), entrambi versantisi nell'emolinfa che bagna gli organi, costituisce senza dubbio un fatto notevole in favore dell' interpretazione dei corpi cardiaci quali glandole a secrezione interna, già da me sin dal 1933 sostenuta.

De Lerma, B. - 1933 - I corpi faringei degli Ortotteri. Prova sicura della esistenza di glandole endocrine negli artropodi. Rend. Acc.Naz.Lincei, vol. 17,p 1105.

__________ 1937 - Osservazioni sul sistema endocrino degli Insetti (Corpora allata e corpi faringei). Arch. Zool. Ital., vol. 24, p.339.

* Nel 1937 io ho descritto, sotto la denominazione di "corpi faringei", i corpi cardiaci dell'idrofilo e di altri insetti, interpretandoli come organi a secrezione interna che sin da allora avvicinavo, in senso isto-fisiologico, a segmenti neuroghiandolari di organi ~~di organi~~ endocrini dei vertebrati (richiamavo in particolare la post-ipofisi). A tale lavoro rinvio per i dati riguardanti la topografia e la tessitura istologica dei c.cardiaci dell'idrofilo.

No.

L. M. Passano

(Yale University, New Haven, Connecticut, USA)

Phase microscopic observations of the neurosecretory product of the crustacean X-organ *

The X-organ of the crab eyestalk consists of a cluster of large neurons, axons of which run together to form the X-organ sinus gland tract. At the end of the tract these, and other axons which join it, enlarge amid a framework of connective tissue. The axonal endings are arranged around a membrane-lined blood sinus and make up the sinus gland. Secretory products appear in the perikaryon of these X-organ neurons and can be traced along the internum of their axons to the enlarged endings in the sinus gland, where material is apparently discharged into the circulation. These facts have been demonstrated histologically, while the production of hormonally active material has been demonstrated experimentally (Passano, 1953); thus the X-organ (specifically the "pars ganglionaris X-organi" portion of the X-organ, following the nomenclature of Carlisle and Passano, 1953) is a neurosecretory endocrine gland.

It has been known for some time (Brown and Cunningham, 1939) that the sinus gland and its adjacent "nerve" have a distinctive whitish opalescence while living. Recently, it has been found that a bluish-white color is also characteristic of the large X-organ cell bodies (Passano, 1951 a). In *Sesarma (Sesarma) reticulatum* (Say) the X-organ consists of a cluster of large, apparently monopolar neurons embedded in the midst of small ganglionic

---

* An abstract of a portion of a dissertation presented for the degree of Doctor of Philosophy in Yale University, June, 1952.

cell bodies in a more or less compact cluster. The individual cell bodies may be 40 microns in diameter and their nuclei are among the largest found in the crab nervous system, although small in comparison to the cell volume. The living nucleus has a lower refractive index (R.I.) than the surround and is quite homogeneous in appearance. There is usually a single subcentrally located nucleolus of higher R.I. The nucleus is spherical with a definite boundary, its shape being emphasized by rows of mitochondria arranged around its periphery with a characteristic "ear-of-wheat" appearance. No variations of nuclear morphology suggestive of a direct secretory role have been observed.

The cytoplasm of the neurosecretory cell, including the axoplasm, contains a great many mitochondria (R.I. higher than the surrounding material). When the cell body is filled with inclusions these bodies are most obvious at the cellular periphery.

Three forms of secretory inclusions have been found in these X-organ cells. By use of dark field illumination it is easily demonstrable that the bluish-white color of these cells is due to highly refractive inclusions in the cytoplasm, axon hillock and axon. These inclusions were studied and photographed both from teased squashes and (with vertical incident light illumination) from the surface of the undamaged, isolated medulla terminalis. They are 2 to 4 microns in diameter and have been termed "spheroid systems" since both dark field and phase microscopic studies have shown that they are made up of a number of small granules, estimated to be 0.3 micron in diameter. These latter granules are of lower R.I. than their surround, and have been tentatively identified as occurring both in the spheroid systems and "free" in the cytoplasm and axoplasm. These secretory granules are not mitochondria (as shown by their R.I.); however mitochondria or similar appearing bodies are often found around the spheroid systems.

Many X-organ neurosecretory cells have their cytoplasm filled with spheroid systems, but in some cells only a few were to be found.

Occasionally in the perikaryon, and characteristically in the axon, large inclusions consisting of a number of spheroid systems were found. These masses of material are spherical in the cell body (and in squashes made from the sinus gland) but elliptical in the axon, which bulges around them. Particularly in the distal portions of the X-organ sinus gland tract (close to the sinus gland) the major part of the characteristic bluish-white color is due to these large inclusions. They are the main inclusions seen with phase when axons of this region are examined. These large inclusions, approximately 7 microns in width by 13 microns in length, appear to possess a definite limiting membrane. They are often lost in routine histological treatment, leaving clear spaces in the axons, but they can be shown as apparently homogeneous masses by the chrome hematoxylin phloxine method. They are fixed and darkened in situ by osmic tetraoxide fixatives.

In summary: one secretory product of the large neurosecretory cells of the crab X-organ (cells experimentally shown to be the major if not the sole site of the molt inhibiting hormone; Passano, 1953) consists of small granules found in the perikaryon, axons and enlarged axonal endings. These granules appear to make up spheroid systems, 2 to 4 microns in diameter, which give the perikaryon and proximal portion of the axons their characteristic appearance in life. The spheroid systems may further coalesce into or make up the large inclusions of these regions of the neurosecretory system. They are especially characteristic of the distal portions of the axons.

It is not possible at this time to equate these observed manifestations

of secretory activity to actual hormonal material, although if not the same thing, they are clearly closely associated in expected concentrations and mode of transport. It is believed, however, that the small granules observed in living cells are those which stain with hematoxylin in the CHP method, that the spheroid system's "structure" is disturbed but not entirely destroyed by usual histological methods, and that the phloxinophilic large masses of the distal portion of these axons are the stained equivalent of the large inclusions seen in living material.

Editor's note:

In a paper entitled "A study of the spheroid system of sympathetic neurones with special reference to the problem of neurosecretion" (Quart. J. Micr. Sci. Vol. 89, 333, 1948) Thomas uses the term spheroid system to mean Golgi bodies. Obviously Passano refers to neurosecretory substance when he speaks of spheroid systems in his abstract. The use of the term in a different sense by the two authors is here pointed out in order to prevent misunderstandings.

No.

Talbot H. Waterman & Masashi Enami

(Yale University, New Haven, Conn., U.S.A. and Hiroshima University, Fukuyama, Japan)

Neurosecretion in the lateral rudimentary eye of Tachypleus, a xiphosuran *

The so-called lateral rudimentary eye of Xiphosurans is a structure of unknown function lying central to the posterior retinal margin of the compound eye. In both of the species so far studied, *Limulus polyphemus* (L.) and *Tachypleus tridentatus* Leach, it consists of two components: a mass of connective tissue filled with white pigment granules and a number of neurosensory cells superficially imbedded in this white body. The neuron somata are large and resemble somewhat the clusters of photoreceptor cells in the retina of the xiphosuran median eye. Their axons run forward in two thin strands which, just anterior to the eye, join the optic nerve proper on its long course to the forebrain.

Examination of appropriately stained sections indicates that these neurosensory cells give evidence of secretory activity in a cycle which may be reconstructed as follows. First an aggregation of mitochondria occurs in a particular area of the cytoplasm. In this region a special ground substance is formed in which basophilic granules and droplets of neurosecretory material are elaborated. These specialized products of the ground substance then move

---

* This research was aided by Contract NR 163-091 between the Office of Naval Research and Yale University and by a grant from the Pacific Science Board of the National Research Council.

out into the surrounding cytoplasm. After that the ground substance itself becomes reduced in mass and finally is replaced by a vacuole. At this point the two specialized products of the neuron soma are discharged into the surrounding white pigmented connective tissue stroma as the last step in the cycle.

No evidence is present that these secretory materials move down the axons of the cells concerned. But since the whole region of this white body is well vascularized, the transport of the basophilic granules and the neurosecretory droplets to their site of action could be accounted for in this way. Thus the data provide evidence for an endocrine system comparable in certain respects to the x-organ sinus gland complex of decapod crustaceans or the intercerebralis corpus cardiacum system of insects. In the xiphosuran case the method of discharge and storage of the neurosecretory materials differs from these others in being directly from the cell body to surrounding tissues rather than via the axons of the neurons.

Exploratory experiments in Limulus have indicated that the rudimentary eye is photosensitive although action potentials have not with certainty been recorded from its fibers. The evidence is adequate, nevertheless, to characterize the secretory neurons as neurosensory. Furthermore, aqueous extracts of these organs in Tachypleus tridentatus were found to have chromatophorotropic effects on the red and vermillion pigment cells of juvenile specimens of the crab Sesarma hematocheir although previous similar tests of Limulus extracts on the fiddler crab, Uca pugnax (Waterman and Passano, 1950, unpublished) did not demonstrate any effects on chromatophores. Current experiments are designed to determine the effects of rudimentary eye removal and to record electrophysiologically the photosensitivity of

this organ. The possibility of finding here some correlation between long term regulatory phenomena and light exposure as in the case of certain vertebrate pituitary and pineal systems is a stimulating one.

This material was presented as a demonstration by Professor Enami. The journal in which the full account will be published by the authors has not yet been decided upon.

No.

D. B. Carlisle

(The Marine Biological Laboratory, Plymouth, England)

The X organ-sinus gland complex, somatotropin, the ovarian inhibiting hormone and sex-reversal in *Lysmata* (*Natantia*, *Hippolytidae*).

Moulting, ovarian development and sex-reversal in the crustacean *Lysmata* are so closely interconnected that an experimental approach to the study of either of the latter phenomena must take into its scope a consideration of the other two.

The moult accelerating hormone (somatotropin, ST) is active upon injection and when taken orally. It is found in the brain and in the thoracic ganglion as well as in the eyestalks and seems to be confined to nervous tissue. Within the eyestalk it is found in both parts of the X organ (*pars ganglionaris* and *pars distalis*), but not in the sinus gland nor in the distal ganglia. In living dissected eyestalks of *Dromia* and of *Lysmata* it is possible to see a slow migration of globules along the X organ connective towards the *pars distalis*. While this movement continues the axoplasm is birefringent, but this birefringence disappears when the movement stops. The migratory globules are probably represented in histological sections by chrome-haematoxylin positive and phloxinophilic elements in the X organ and X organ connective. These elements are much depleted after an injection of an extract containing ST. They are probably cytological manifestations of the hormone, which appears to be formed in the *pars ganglionaris X organi*, transported along inside the axons of the X organ connective to the *pars distalis X organi* and there stored inside the enlarged nerve endings until it is finally released. It is also formed in neurosecretory cells of the

brain and of the thoracic ganglion and probably passed from there along the axons which terminate in the *pars distalis*.

The ovarian inhibiting hormone is effective upon injection of eyestalk extracts in stopping ovarian development in intact *Lysmata*. Removal of eyestalks has the reverse effect in stimulating ovarian development as measured by weight and by advance of vitellogenesis. The injection of eyestalk extracts into eyestalkless animals reduces the heightened rate of ovarian development to normal levels. The hormone is found in the *pars ganglionaris X organi* and in the sinus gland, but not in the *pars distalis X organi* nor in the distal ganglia of the eyestalk. It is not active when taken orally. Probably it is produced in neurosecretory cells of the *pars ganglionaris* and passed along the axons of the sinus gland tract to the sinus gland where it is stored and finally released. The ovarian inhibiting hormone is probably the agent responsible for the inhibition of sex reversal, for the rate of sex-reversal is strictly correlated with the rate of ovarian development, both under natural conditions and through all experimental manipulations.

The moult accelerating hormone is clearly distinct from the moult inhibiting hormone and from the ovarian inhibiting hormone, for it is released at a different organ, - the *pars distalis X organi*, instead of the sinus gland - and it is possible to obtain an extract containing moult accelerating hormone but no ovarian inhibiting hormone and *vice versa*. Although the ovarian inhibiting and moult inhibiting hormones are both released at the sinus gland and may come from the same cells of the *pars ganglionaris*, they too seem to be distinct substances, for the time of greatest ovarian development in *Lysmata* is the time of the lowest moult-rate - midwinter - while the time of fastest moult-rate - August - is a period at which the ovary is regressing.

There is thus no correlation between the degree of inhibition of moulting and that of inhibition of ovarian development, so that the same agent cannot be responsible for both.

The neurosecretory material undergoes a definite change as it passes along the axons of the sinus gland tract and X organ connective. The globules and granules agglomerate into larger masses as they pass along, and they cease to be chrome-haematoxylin positive and become phloxinophilic instead.

The complete report will appear in Pubbl. Staz. zool. Napoli, Vol. 24, 1953.